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JOHN MERLE COULTER

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P. 491, line 4 from bottom, for accitillo read aceitillo.
P. 494, line 4 from bottom, for *B. incisa* (L.) G. Don read *B. incisa* (Ker) G. Don.

Vol. LVII

P. 308, footnote, for LXII read LXXII.
P. 415, line 12 from top, for ongam read longam.
P. 417, line 9 from top, for dela read de la.
P. 424, line 18 from top, insert comma after lata; and for petiole read petiolo.
P. 424, line 23 from top, for insertionen read insertionem; and omit comma after insertionem.
P. 430, legend of Fig. 3, for petals read perianth; and for sepals read stamens.

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Editor: JOHN M. COULTER

JANUARY 1914

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THE
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JANUARY 1914

THE DEVELOPMENT OF MAGNOLIA AND LIRIODENDRON, INCLUDING A DISCUSSION OF THE PRIMITIVENESS OF THE MAGNOLIACEAE[1]

WILLIS EDGAR MANEVAL

(WITH PLATES I–III)

The recently announced theories of ARBER and PARKIN, especially as developed in their paper "On the origin of angiosperms" (**2**), make it desirable that more detailed work be done on the Magnoliaceae and related groups. Besides, as the embryo sac of only one species of the Magnoliaceae, *Drimys Winteri* (**29**), has ever been studied, it seemed probable that an investigation of other genera of this family, from this point of view, might be of value in several ways, but especially in furnishing either positive or negative evidence concerning the primitiveness of this family.

In the present study of *Magnolia virginiana* L. and *Liriodendron Tulipifera* L. two objects have been kept in mind: (1) the determination of the course of development of the sporogenous tissues and of the mature gametophytes, and also the examination of certain points concerning the gross structure and anatomy of these forms; (2) a consideration of the primitiveness of the Magnoliaceae on the basis of the evidence gained by such investigation, and of present prevailing theories respecting the origin of angiosperms.

The collection of material was begun in December of 1910 and was continued at intervals of generally two or three weeks until

[1] Contribution from the Botanical Laboratory of the Johns Hopkins University, no. 34.

September 1911. Material for the study of *Liriodendron* was obtained at Homewood, the new site of the Johns Hopkins University, and that for *Magnolia* in a swampy region at Glen Burnie, Maryland, a typical habitat for the so-called swamp magnolia. Most satisfactory results were obtained by using a chromacetic solution for fixing, although acetic alcohol worked well also. The principal stains used for sporogenous tissues and young embryo sacs were iron-hematoxylin and orange G; and for older sacs safranin and Delafield's hematoxylin, or for embryos hematoxylin alone.

The writer is greatly indebted to Professor DUNCAN S. JOHNSON, and desires to express thanks for many suggestions and much helpful criticism during the progress of the work.

As the course of development is very similar in *Magnolia* and *Liriodendron*, in the following description attention will be directed mainly to the former, differences between the two species being pointed out where they occur.

The stamens of *Magnolia* and *Liriodendron* have elongated anthers on short filaments, the connectives extending beyond the anthers. The anthers contain four locules, with walls of 3 or 4 layers of cells, and dehisce longitudinally. In *Magnolia* the sporogenous tissue in the stamens is already differentiated early in December (fig. 15), and in *Liriodendron* early in January. Little change occurs in this tissue until March, when the sporogenous cells enlarge considerably and divide. The nuclei of the microspore mother cells are found in synapsis from April 20 to May 1 (fig. 16). The first mitosis in both species usually occurs during the first week in May (fig. 2), and tetrads are developed by the simultaneous method about May 10 (fig. 18).

After the first and second mitosis of the nuclei of the microspore mother cells the number of chromosomes is 19 (figs. 3 and 19). The actual number in the microspore mother cells, or in the vegetative tissues, was not determined, but is much greater than 19; therefore, as the usual heterotypic division occurs in the microspore mother cells, and after this division a smaller number of chromosomes than before is present, there can be little doubt that in microspore formation reduction takes place, and that the x and $2x$ generations are characterized respectively by 19 and 38 chro-

mosomes. Neither STRASBURGER (29) nor ANDREWS (1) determined the exact number of chromosomes in the forms studied by them.

The mature pollen grains are oval in shape, with rather thick walls. In the case of *Magnolia* they are binucleate (fig. 21), while those of *Liriodendron* are two-celled (fig. 4). This condition is found in the anthers before dehiscence occurs, which, in the latter species, is between May 15 and 18, and in the former about May 30.

In early stages of development it is evident that the tapetum arises from the sporogenous tissue (fig. 15), later differing from it especially in size of cells and nuclei (fig. 17). Still later, when tetrads are forming, the tapetum consists of 2 or 3 layers of large, binucleate cells lining the walls of the loculi (fig. 1). Finally, as the pollen grains mature, the tapetum gradually disappears, but there is no evidence of migration of its nuclei among the developing pollen grains (fig. 18). The walls of the loculi consist of 3 or 4 layers of cells. As the anthers mature, the subepidermal layer of cells becomes differentiated into the usual type of cells which are active in dehiscence.

The ovules in both *Magnolia* and *Liriodendron* are marginal in origin and anatropous, but there is considerable difference in the time of initiation of these organs. It occurs in the former as early as the first two weeks in December or earlier, while in the latter not until after the middle of March. Early in April, however, the condition attained is about the same in both, and the two develop at the same rate until the completion of the embryo sac. The first rudiment of the inner integument appears when the megaspore mother cell is well differentiated (April 20), and soon afterward the outer integument is initiated (fig. 5). From the archesporial cell (fig. 22) a tapetal cell is cut off (fig. 23), apparently in both species, and the mother cell soon becomes deeply buried within the nucellus (fig. 24). By two successive divisions of the megaspore mother cell 4 megaspores are formed (figs. 6, 7, 25, 26, 27), the innermost of these in each case being functional, while the others degenerate. By the time the 4 megaspores have developed the innermost one comes to lie very deep within the nucellus (fig. 7).

The development of the embryo sac is normal throughout.

Even in the binucleate condition the sac is considerably elongated (fig. 28); it continues to grow more in length than in breadth; finally it becomes slightly curved and also somewhat enlarged at the ends. The tetranucleate sac is shown in fig. 29. The mature sac is of the ordinary type, containing an egg and two beaked synergids at the micropylar end, three antipodals at the opposite end, and two endosperm nuclei somewhat above the middle (figs. 8, 9, 10, 30).

While fertilization was not observed in either species, there can be little doubt of its occurrence in both. The evidence for this is that when material of a certain age is examined the remains of the pollen tube are invariably found in the micropyle and also within the embryo sac (fig. 31).

In all the sacs of *Magnolia* in which the polar nuclei were found, partial fusion had already occurred (fig. 30). After fusion the endosperm nucleus moves close to the egg apparatus, and then, before division of the (presumably fertilized) egg, the first division of the fusion nucleus takes place. At this division a wall is formed transverse to the long axis of the embryo sac (fig. 31), hence the endosperm is cellular from the start, differing from *Drimys* (**29**) in this respect. Both of the resulting cells participate in the formation of the endosperm, that part of the sac surrounding the egg becoming filled with endosperm much more rapidly than the antipodal end. In early stages the endosperm is quite compact (fig. 32), but later the cells seem to enlarge; finally, in the mature condition, the nucellar tissue disappears completely and an abundant supply of compact endosperm fills the seed.

In the development of the embryo of *Magnolia* the first division is transverse, the second longitudinal to the long axis of the embryo sac (fig. 33). A second longitudinal wall separates the young embryo into octants (fig. 34), and soon thereafter the divisions apparently become quite irregular (fig. 35). There is much difference in the form of different embryos of approximately the same age, some being nearly globular (fig. 35) and others considerably elongated. A well defined suspensor (fig. 36) appears somewhat late and may persist until the embryo is mature. The cotyledons are initiated after the embryo has enlarged considerably.

They appear simultaneously and are independent from the start, there being no evidence of a pseudo-monocotyledonous habit such as occurs in various other members of the Ranales. In the mature seed the embryo is typically dicotyledonous, with a short radicle and well developed hypocotyl and cotyledons (fig. 37).

The seed coats of *Magnolia* have been described by different botanists, probably the earliest correct description being that given by ASA GRAY (10). In the mature seed the outer integument is differentiated into two layers, an outer fleshy one well filled with oil receptacles, and an inner stony layer of bony hardness. The inner integument forms only a thin layer in the ripe seed. After dehiscence of the carpels the seeds remain suspended a few days by means of an elastic thread formed from the spiral thickening bands of the xylem elements of the raphe. Finally the threads may be broken and the seeds fall to the ground; or sometimes the entire cones with most of the seeds still attached are shed. This shedding of the cones results from a break across the base of the peduncle, but without the formation of a definite absciss layer.

In the case of *Liriodendron* the development of the endosperm and embryo were not investigated.

Before discussing the embryo sac and certain other points mentioned above, several features of gross structure and anatomy deserve attention. ARBER and PARKIN (2) define the term flower as "a special form of a type of strobilus common to angiosperms and certain mesozoic plants," and propose to designate it as an anthostrobilus. The anthostrobilus differs from all other strobili in that it is typically amphisporangiate, with the megasporophylls above the microsporophylls on an elongated axis, and below the sporophylls a distinct perianth which is wholly or partially protective. The angiospermous type of anthostrobilus is called a euanthostrobilus, and is believed by them primitively to have possessed among others the following characteristics: a large or indefinite number of parts arranged spirally; ovules orthotropous, several in each ovary, with two integuments; marginal placentation; filaments short, bearing long anthers with the connectives prolonged beyond them; members of the perianth all similar, or more or less differentiated; entomophilous. The flowers of

Magnolia and *Liriodendron* differ from the above type only in having anatropous ovules and two-seeded carpels. In the proanthostrobilus or Bennettitean type of "flower" corresponding parts occur with a similar arrangement. ARBER and PARKIN hold that "its parts are homologous with the carpels, stamens, and perianth of a typical amphisporangiate angiospermous flower." It differs from this, however, "especially in the presence of a seminal pollen-collecting mechanism, and in the form of the microsporophylls," which are decidedly fernlike. More details regarding the proanthostrobilus cannot be included here, but may be found in the well known work of WIELAND (**32**).

The seedling structure of *Liriodendron* has been described by Miss THOMAS (**30**). She finds here a form intermediate between the normal tetrarch and diarch types of transition from cotyledons and hypocotyl to root as found in the Ranales, Rhoedales, and various other dicotyledons. The writer has confirmed Miss THOMAS' descriptions. The peculiarity in *Liriodendron* is that while in the cotyledons and upper part of the hypocotyl the structure is that ordinarily occurring in connection with the tetrarch type of root (figs. 13, 14), lower down in the hypocotyl certain elements disappear so that the root is diarch (fig. 12). On account of lack of material the seedling of *Magnolia* has not yet been studied.

In both gross structure and anatomy *Magnolia* and *Liriodendron* afford many points of similarity, possessing certain characters common to all Magnoliaceae and others which are peculiar to the Magnolieae (**28, 21**). Among the former are woody stems, alternate leaves, secretory cells, a characteristic type of stoma, and more or less abundant endosperm in the seed; the latter include foliar stipules, sclerenchymatous diaphragms in the pith, and the bundles of the petiole more or less fused in an irregular ring.

The arrangement of the vascular bundles in the peduncle of *Magnolia* and in the petioles of *Magnolia* and *Liriodendron* is shown in figs. 11, 38, and 40, and details of the petiolar bundle of *Magnolia* are represented in fig. 39. Considering the Magnoliaceae as a whole, the petiolar bundles are distributed either as in these two species or in the form of a crescent. PARMENTIER (**21**) regards

the different arrangements as correlated with the size of the leaves. WORSDELL (33) finds medullary bundles in the petioles of various species of *Magnolia*, and interprets these as reminiscences of a primitive, more scattered system in their ancestors. He believes also that the somewhat irregular system of bundles in the peduncle of *Liriodendron* and *Magnolia* indicates the same thing. If we accept the view that dicotyledons have been derived from monocotyledons, this explanation might seem more or less plausible; however, on the theory that monocotyledons are secondary, this interpretation could hardly be correct.

Present theories concerning the primitiveness of various types of angiospermous embryo sac will now be discussed. According to the archegonium theory of PORSCH (**22**), the 8-nucleate embryo sac is to be interpreted as the equivalent of two archegonia, a micropylar one represented by the egg apparatus, and a chalazal one represented by the three antipodals. Accepting this view, as has been pointed out by BROWN (3), "we might conceive of the embryo sac of *Peperomia* as really composed of four sacs, each of which gives rise to one archegonium." A similar explanation might hold also for certain other anomalous embryo sacs such as are found, for example, in the Pennaeaceae. PORSCH, however, in his paper considers only the 8-nucleate type.

This theory has been criticized from various standpoints. In the gymnosperms, for instance (3), in all species that form archegonia, the megaspore first produces a non-cellular stage of the gametophyte, and subsequently a cellular stage in all species that form archegonia; and it is in this cellular tissue that the archegonia are initiated and formed. So if we homologize the first two divisions of the ordinary angiospermous embryo sac with the free nuclear divisions of the gymnospermous prothallus, the shifting of the archegonia from the cellular to the non-cellular stage of the prothallus must be explained.

ERNST (9) admits that the archegonium theory would be *sehr bestechend* if within the 8-nucleate embryo sac the two groups of nuclei were always of nearly the same form. The numerous variations from the "normal" type, not only in the form of the egg apparatus and of the antipodal apparatus, but also in the

behavior of the polar nuclei, serve, he believes, better to refute than to support PORSCH's theory.

Other valid criticisms might be offered, but we need not do this here, since, even if we were to accept the theory, the question as to the most primitive type of angiospermous embryo sac would still remain unsolved.

In a recent publication (9) ERNST, after considering the course of development of the ordinary angiospermous embryo sac, as well as of such peculiar types as occur in *Gunnera macrophylla*, *Peperomia pellucida*, *P. hispidula*, and the Pennaeaceae, arrives at conclusions quite different from those published slightly earlier by COULTER (5). According to COULTER, the most important of the five nuclear divisions in the development of the ordinary embryo sac are the first two, which result in the formation of tetrads. COULTER asserts that, so far as we know, if fertilization is to occur later, the reduction divisions are not omitted; and that whether the number of divisions of the embryo sac mother cell is reduced from 5 to 4, or even to 3, the reduction divisions are never omitted. In such forms as *Peperomia*, in spite of the fact that the embryo sac contains 16 nuclei, there are only 4 divisions of the embryo sac mother cell, instead of 5 as in the ordinary 8-nucleate type of embryo sac.

To this view ERNST replies: "Die Entwicklungsvorgänge im Embryosack scheinen mir unabhängig von seiner Entstehung betrachtet werden zu müssen." So ERNST holds that although the omission of tetrad formation is a reduction of the course of development and not a primitive character, it has, nevertheless, no influence on the development of the embryo sac, and that the 5 divisions of the embryo sac mother cell resulting in the 8-nucleate sac belong to two entirely different phases of development; that is, formation of spores, characterized by reduction of chromosomes, and germination of spores, characterized by polarity, number of nuclear divisions, position of nuclei, development of vacuoles, and cell formation.

It is evident that COULTER regards the 16-nucleate embryo sac of such forms as *Peperomia* as derived by a reduction of the divisions of the embryo sac mother cell to 4 instead of 5. ERNST,

on the other hand, sees here an omission of tetrad formation and one more than the usual number of divisions in the germination of the megaspore to the embryo sac. He therefore considers the 16-nucleate sac as an older, or at any rate an independent, form of embryo sac, not derived from the 8-nucleate type. These two views, then, are directly opposed to each other.

With respect to the nature of the 16-nucleate embryo sac of *Peperomia*, CAMPBELL (4) and JOHNSON (15, 16), as is well known, have arrived at opposite conclusions. CAMPBELL says: "*Peperomia*, in regard to the embryo sac, probably represents the most primitive form yet discovered among the angiosperms. . . . . *Peperomia* offers a basis for an explanation of the homologies of the embryo sac." JOHNSON, a little later, after studying several genera of Piperaceae, maintained that the peculiarities in *Peperomia* are of secondary origin. The latter view is supported by BROWN (3) who, in *Peperomia sintenisii* and *P. arifolia*, finds that at the first two divisions of the embryo sac mother cell typical reduction of chromosomes occurs; and also that during these divisions the nuclei are separated by evanescent cell walls. So he concludes that these phenomena seem to indicate that the sac is a compound structure derived from the nuclei of four megaspores, the primary sac nucleus being a mother cell rather than a megaspore.

Reference has been made to COULTER'S view that while the genesis of the ordinary angiospermous embryo sac from the megaspore mother cell involves 5 divisions, the essential part of the process is found in the first two divisions which, so far as we know, are necessary if fertilization is to occur. BROWN (3) maintains that we cannot make chromosome reduction the sole criterion of megaspore formation, and adds as another distinction that while a division giving rise to megaspores is characterized by a cell wall or a cell plate, the first division of a megaspore is not accompanied by a cell plate. Quite lately SMITH (27) has pointed out that this distinction does not hold in the case of *Clintonia*, where, at the first division of the megaspore nucleus, a cell plate appears, and SMITH infers it will not hold for certain other cases.

Sufficient emphasis, it seems to the writer, has never been given to the fact that while the anomalous types of embryo sac are as a

rule of rare occurrence and are distributed among entirely unrelated families, on the other hand, the ordinary 8-nucleate type developed by 5 divisions of the megaspore mother cell occurs in nearly all families of angiosperms from the most primitive to the most specialized. This is significant and is hardly to be explained on any other hypothesis than that the latter type is primitive. We have in the 8-nucleate sac, it seems, a structure of marvelous constancy in development and arrangement of parts, evolved in the course of long ages, the exceptions to which only strengthen the view that it is a primitive type of embryo sac.

Another reason for regarding anomalous types of embryo sac as derived is the variability in the manner of their development. This is especially marked in the case of 16-nucleate sacs, where a peculiar method of development is found in almost every new case discovered. Of course it may be objected that these variations are of minor importance. But even if this were true, it certainly contrasts strikingly with the remarkable uniformity so very general even in minor details of the development of the ordinary angiospermous type, and is a fact to be explained.

Whether or not we consider the first 4 nuclei produced by division of the embryo sac mother cell as megaspores, and this view seems reasonable in most cases, is probably of less importance than many have believed. The genesis of the angiospermous sporophyte, aside from the exceptional cases such as those involving apogamy and budding, always begins with a perfectly definite structure, the embryo sac mother cell. If fertilization is to occur later, then, without exception, reduction of chromosomes takes place at the first two divisions of this cell. Now, considering parthenogenesis as a secondary phenomenon, we see that in the development of all normal embryo sacs (that is, those capable of being fertilized), whatever their type of mature structure, there is this common feature in development. After the reduction divisions the embryo sac may develop as the product of one-fourth, one-half, or all of the four reduced nuclei derived from the original mother cell. The ordinary 8-nucleate type develops as the product of one-fourth of these nuclei by three successive divisions; the sac of *Cypripedium* (**20**) develops as the product of one-half of these

nuclei by one division; that of *Lilium* as the product of all of these nuclei by one division; and that of *Peperomia* from all of these nuclei by two divisions. The three latter cases clearly show an abbreviation in the course of development of the gametophyte, whether we regard that course as beginning with the embryo sac mother cell or after chromosome reduction.

That we cannot make chromosome behavior the sole criterion for distinguishing sporophyte and gametophyte is doubtless true. This is evident from chromosome behavior in cases of parthenogenesis such as occur in *Alchemilla* (19), and in the numerous instances of apogamy and apospory among both pteridophytes and spermatophytes. However, it is probable that no one has ever dreamed of making such unusual phenomena the basis of any theory of the nature or phylogeny of the angiospermous embryo sac. These phenomena undoubtedly are secondary. Hence the conception that chromosome behavior is the most important criterion, at least in all cases where fertilization occurs, seems well founded. So if abbreviation in the developmental history of the gametophyte in angiosperms expresses an evolutionary tendency which can be traced back as far as the gametophyte of the pteridophytes, then anomalous embryo sacs are secondary rather than primitive types.

If now the embryo sacs of those Magnoliaceae thus far investigated are considered, no clue is discovered in their development or structure as to the primitiveness of the group; and this for the simple reason that, although we regard this type as the most primitive among angiosperms, yet the same type is the common one among all angiosperms. Moreover, whatever theory we accept, the past study of the embryo sac has served mainly to emphasize the vast difference between angiosperms and lower groups of plants in this respect, and so to increase rather than to bridge the wide gap between them. It would seem then that if the problem of the origin of angiosperms is to be solved this must come about principally as a result of investigations of other features than the embryo sac.

During the last two decades the amount of purely descriptive literature dealing with embryo sacs has grown to huge proportions,

but the different theories as to which type is primitive have not been reconciled. If, however, anomalous types of angiospermous embryo sacs are secondary and not primitive, as seems most reasonable, then the causes of these secondary modes of development should be investigated. This is obviously a problem presenting serious difficulties, yet they are probably not insurmountable. A key to the situation may probably be found by considering the possibility that there is some relation between anomalous types of embryo sac development and peculiar environmental conditions. This has been suggested, for example, by JOHNSON (15).

Since the strobilus theory of ARBER and PARKIN (2) is based so largely on a comparison of the flower (euanthostrobilus) of angiosperms and the proanthostrobilus of the Bennettitales, we must refer to certain views as to the nature of these structures. ARBER and PARKIN, agreeing with HALLIER (11) and SENN (26), consider the amphisporangiate flower of *Magnolia* and *Liriodendron* as made up of sporophylls and perianth borne directly on the main axis of the floral shoot rather than as a compound structure. This they regard as the most primitive type of angiospermous flower, reproducing the essential features of the Bennettitean strobilus. WETTSTEIN (31) and others, on the contrary, think that the primitive type is to be sought for among the monosporangiate Apetalae, and that the angiospermous fructification is a reduced inflorescence, derived from that of the gymnosperms. LIGNIER (17), at least, interprets the Bennettitean strobilus also as a compound structure, an inflorescence. It is clear that conclusions as to the primitiveness of different groups, as well as to the origin of angiosperms as a whole, will vary according to which of these views is accepted.

The greatest differences between the two theories have been indicated. Whether the primitive angiospermous flower was anemophilous or entomophilous is perhaps of relatively minor importance, yet this question also is involved in both of these theories. It would seem natural to imagine anemophily as the method of pollination among primitive angiosperms, yet if entomophily has played as important a rôle as many suppose in their evolution, from their very origin, then the latter must be primitive for this group.

Thus far the monocotyledons have been left out of account in looking for primitive angiosperms. Let us now turn to this group.

The remarkable uniformity in the development and mature condition of the male and female gametophytes of monocotyledons and dicotyledons has been brought forth repeatedly as the strongest argument in favor of a monophyletic theory. In spite of objections such as the claim that "similarity in structure may be the outgrowth of the changes that resulted in the evolution of seeds" (**6**), it seems that we are far from the point of even thinking of abandoning this as well-nigh irrefutable evidence of close genetic relationship between the two great classes of angiosperms.

Within the last few years the striking similarity in the seedling structure of dicotyledons and monocotyledons has been demonstrated in many forms. Although the generalization that "ontogeny repeats phylogeny" has likely been overworked, yet if there is anything at all in this rule, then the evidence from seedling structure deserves its full share of consideration.

In favor of the view that the two groups have originated independently are the differences in their anatomy, and in the development and mature condition of the embryo. As a result of recent study, however, we learn that anatomically the structure of seedlings in particular, but also of mature individuals in the two groups, offers many striking points of resemblance. Other differences, generally regarded as secondary in importance, are seen in the venation of the leaves, in the grandifoliate as opposed to the parvifoliate habit, and in floral symmetry.

The principal resemblances and differences between monocotyledons and dicotyledons on which present phyletic theories are based have been mentioned. In reviewing these theories we may recall the fact that while according to most if not all monophyletic theories proposed until recently, dicotyledons were assumed to be derived from monocotyledons, students of phylogeny today quite generally hold the opposite view. WORSDELL (**33**), however, is still inclined to the view that monocotyledons rather than dicotyledons are primitive. He believes that "angiosperms have developed directly from an ancestor belonging to the bryophytic level, and that they

have not come from either gymnosperms, pteridosperms, or ferns." His conclusion is based mainly on the following insufficiently substantiated assumptions: that taking the vegetable kingdom as a whole, the grandifoliate habit is primitive, the parvifoliate derived; that the appearance of two cotyledons in dicotyledons is illusive, there really being only one which is deeply bifurcated; that in the monocotyledonous seedling there is no room for the scattered arrangement of the bundles, so we cannot on account of its absence here conclude that the scattered condition has been derived from a vascular cylinder. These points cannot be discussed here; it suffices to say that the criticism that this view assumes entirely too much seems fair. Besides, the evidence from fossils is entirely against it, the general conclusion of paleobotanists being that from the Bryophyta no higher forms have ever evolved. SCOTT (**25**), touching this point, says: "neither among living nor fossil plants has any indication of a structure intermediate between the plant of a vascular cryptogam and the fruit (sporophyte) of a bryophyte ever been discovered."

The view that monocotyledons have been derived from dicotyledons, doubtless at a rather early period in the history of angiosperms, and possibly by branching from several points along the dicotyledonous line, has been much strengthened by the researches of the last 15 years. Miss SARGANT (**24**) regards the common characters of monocotyledons and dicotyledons as too numerous and uniform to have been acquired independently, and emphasizes the fact that angiosperms are especially unique with respect to their flowers, carpels, and endosperm. The attainment of practical identity in the "germination of the embryo sac and the history of the endosperm" by independent evolution among monocotyledons and dicotyledons "would require a series of coincidences," she says, "so improbable as to be inconceivable." With respect to the flower, Miss SARGANT agrees with the view of ARBER and PARKIN given elsewhere.

Probably Miss SARGANT'S most important contribution to the monophyletic theory is based on anatomical investigations. The presence or absence of a cambium seems to account largely for the difference in detail between monocotyledonous and dicotyledonous

stems, hence the great importance of any evidence as to its presence among primitive angiosperms. That a true cambium develops in certain monocotyledons (*Gloriosa*, **23**) is now well known; but especially significant is the fact that while the structure of the mature stem of monocotyledons and dicotyledons generally varies greatly, the primary structure of the dicotyledonous stem, which is the same in seedlings and mature plants, is also frequent in monocotyledonous seedlings. So in the light of this evidence and also of the fact that a cambium is usually present among living gymnosperms and extinct vascular cryptogams, and is universally present, so far as known, among extinct gymnosperms, the view that primitive angiosperms possessed a cambium seems well founded.

The argument for most of Miss SARGANT'S other views cannot be included here, yet one other conclusion should be mentioned. Since among gymnosperms monocotyledonous forms are unknown, and the dicotyledonous condition prevails, one would naturally presume that the embryo of primitive angiosperms was dicotyledonous; besides, the embryological development of angiosperms points in the same direction. So the general conclusion is that monocotyledony is secondary, being the result of a fusion of two cotyledons.

ARBER and PARKIN in discussing the origin of angiosperms hold "that monocotyledons branched off from the main angiospermous line, that is, dicotyledons, at a very early period." Since the embryo of *Bennettites* was dicotyledonous, they regard the Hemiangiospermae as dicotyledonous also, and so conclude that the monocotyledonous type is the less primitive one. In their opinion Miss SARGANT'S explanation of the monocotyledonous embryo is the best yet offered. They also recognize the need of accounting for the origin of the monocotyledonous habit.

For a number of years the so-called anomalous dicotyledons have attracted students in the hope that knowledge of their embryos and anatomy would aid in solving phylogenetic problems. One of the general conclusions of such investigations is that the anomalous characters are secondary and not primitive features. MOTTIER (**18**), for example, says, "it is probably true without

exception that dicotyledonous plants possessing anomalous embryos are either partially or wholly geophilous in habit, having stems either in the form of a rhizome, tuber, or a short, squat axis."

That anomalies not only in embryology and anatomy, but even in embryo sacs, depend largely on environmental conditions has been suggested in the past (JOHNSON **15**), and seems continually to be receiving wider attention. HILL (**14**), for example, has discovered in the Andes, Central America, and Mexico a few geophilous species of *Peperomia* which are of great interest. Although their seedlings are described as possessing all the external characters of monocotyledons, yet they are true dicotyledons, but the cotyledons exhibit a marked division of labor, one serving for absorption, the other for photosynthesis. That these species are true dicotyledons is shown by the structure of the seed; the presence of stomata on the lamina of the absorbent cotyledon; persistence of the primary root for some time after the formation of the bulb; and the vascular structure of the seedling. Moreover, most of the members of the genus, containing some 400 species, are normally dicotyledonous. The peculiar habit of these few species is therefore interpreted as due to xerophytic conditions, resulting in the assumption of the geophilous habit, accompanied by formation of bulbs or tubers, and finally affecting even the embryonic structure of the plants so that the division of labor referred to above has resulted. Although HILL opposes Miss SARGANT'S theory as to the origin of the single cotyledon of monocotyledons, it is worthy of note that he attributes the anomalies in *Peperomia* to the geophilous habit. This is the same cause that other workers have assigned for the pseudo-monocotyledonous habit, anomalous stem structure, and so on, in the case of various other dicotyledons.

Now if it is conceivable that a pseudo-monocotyledonous habit has arisen in different ways among dicotyledons, then the possibility of the same thing happening among monocotyledons presents itself, especially if monocotyledons have branched off at several points along the dicotyledonous line. Indeed, attempts at a causal explanation of the origin of monocotyledons from dicotyledons have actually been made, one of the most important of these being HENSLOW'S theory (**12**).

This theory is founded mainly on the large number of coincidences among both dicotyledonous and monocotyledonous aquatic plants, and on the fact that all terrestrial monocotyledons exhibit the same coincidences. HENSLOW, as well as many others, regards the monocotyledons as degenerate (when compared with dicotyledons), although there is not always agreement as to the cause of degeneracy. HENSLOW himself believes an aquatic habit has been the principal cause, and points to a number of characteristics of monocotyledons and dicotyledons that he considers the result of adaptation to a moist or aquatic environment. Among these peculiarities are large size of leaves, water storage organs, the pseudo-monocotyledonous condition of certain dicotyledons, early loss of primary root, and "endogenous" arrangement of cauline bundles. HENSLOW remarks: "In the title of my first paper in 1892 (13), I used the word 'theory,' but . . . . I feel justified in abandoning the term; for I would maintain that the conclusion has passed the stage of hypothesis and probability only, to that of a *demonstrated fact*." While it is likely that very few of us would be willing to subscribe to this conclusion, yet all must agree that such considerations are extremely suggestive, and indicate a line of work that is promising, especially if taken up experimentally.

To multiply examples would probably not add to the force of the argument. We see that various competent workers attribute anomalies among dicotyledons to a geophilous habit, response either to xerophytic or to hydrophytic conditions. Such responses result in structural peculiarities in stems, formation of various types of underground stems, a pseudo-monocotyledonous habit, or division of labor among the cotyledons. When we turn to the monocotyledons, we find these peculiarities duplicated, but as a rule they are intensified. That their production is related to environment seems clearer in the case of dicotyledons than of monocotyledons, no doubt because many monocotyledons at present live where the prevailing conditions do not seem to necessitate geophily. The persistence of these peculiarities in such environments may be interpreted as retention of past characters.

It seems then that, on account of many similarities between

monocotyledons and anomalous dicotyledons, HENSLOW'S conclusion that the former have been derived from the latter as a response to the same factors that determined the geophilous habit is reasonable, at least, as a working hypothesis. Failure to recognize the fact, however, that geophily may express itself variously has sometimes led to disagreement in theories where none actually exists. This appears especially in discussions of the origin of the monocotyledonous from the dicotyledonous habit, it being held that monocotyledony has arisen in only one way. There are at least three plausible theories concerning the method by which this may have occurred: first, by suppression of one of two cotyledons; next, by fusion of two cotyledons; and, finally, by a division of labor between two cotyledons. Now since we find a difference in the behavior of the cotyledons in certain anomalous dicotyledons, it is entirely probable that the same thing has happened in the origin of monocotyledons from dicotyledons, so much the more so if there is more than one monocotyledonous branch from the primitive dicotyledonous stock.

While, as has been shown, the most generally accepted view is that angiosperms are monophyletic we must also remember the possibility of a diphyletic origin. COULTER (**6**) has expressed his view on this point as follows: "In our judgment the evidence is strongly in favor of the independent origin of the two groups, which have attained practically the same advancement in the essential morphological structures, but are very diverse in their more superficial features. Their great distinctness now indicates either that they were always distinct or that they originated from forms that were really proangiosperms and neither monocotyledons nor dicotyledons." Those who hold this view will have to explain more satisfactorily than has been done the similarity between monocotyledons and dicotyledons in gametophytic development and in seedling structure; the general similarity between monocotyledons and anomalous dicotyledons; and the evidence that primitive angiosperms possessed a cambium and were dicotyledonous. The monophyletic theory has been strongly reinforced in recent years and the writer finds it more acceptable than the diphyletic, but more evidence is needed before it can be unreservedly accepted.

It is evident then, from the above considerations, that we regard the Magnoliaceae, since they belong to the more primitive group of angiosperms, as more primitive than the monocotyledons. Let us next consider certain theories relative to the primitiveness of various features among the dicotyledons themselves.

Present theories on this subject are fairly well represented by the views of WETTSTEIN (31) on the one hand and those of ARBER and PARKIN (2) on the other. Both believe that monocotyledons have been derived from dicotyledons. WETTSTEIN holds, because of similarity in cotyledons, stem, floral structure, and reduction of the primary root, that monocotyledons have been derived from the Polycarpicae. He also points out that while we may think of derivation of one cotyledon from two, the reduction of a primary root, and so on, the opposite would not seem possible under any circumstances. So he concludes that we must turn to the dicotyledons in considering the phylogeny of angiosperms, but disagrees entirely with many as to which dicotyledons are most primitive.

Both theories derive angiosperms from gymnosperms. According to WETTSTEIN, primitive angiosperms should present among others the following characteristics: prevalence of woody plants and absence of vessels in the vascular bundles; prevalence of monosporangiate flowers, with either no perianth or one of simple structure; prevalence of anemophily. These are gymnospermous characters, and WETTSTEIN holds that we should regard that group of angiosperms as most primitive which exhibits these characters developed in high degree. ARBER and PARKIN (2), HALLIER (11), and others give a much longer list of characters which they believe are primitive. According to their views, amphisporangiate, actinomorphic flowers, with elongated axes bearing numerous free, spirally arranged floral parts, are primitive. Such flowers also possess a well developed, undifferentiated perianth and are entomophilous. Besides, primitive angiosperms are dicotyledonous, have small embryos and abundant endosperm, are treelike, and lack true vessels among autophytic species.

If it is granted that all the essential characters that may be regarded as primitive have been included in these lists, then it is evident that the great differences between the two theories relate

to but a few points, points which involve, however, no end of difficulty. The two theories would apparently be reduced to one if we could say which of the following are primitive: monosporangiate or amphisporangiate flowers; presence or absence of a perianth; anemophily or entomophily. Of possibly less importance is the question whether the angiospermous flower is a modified simple (that is, unbranched) shoot or a modified inflorescence. There is agreement as to the primitiveness of such characters as actinomorphy, freedom of floral parts, dicotyledony, lack of true vessels in the vascular strands, and prevalence of woody plants. So it seems important to discuss the differences cited.

The fact that certain characters are common to all or nearly all gymnosperms seems in many instances to be one of the strongest reasons for regarding those characters as primitive if they occur at all among angiosperms. Among these supposedly primitive characters are dicotyledony, prevalence of woody plants, and absence of tracheae in the conducting strands. For the same reason we might conclude that primitive angiosperms were anemophilous and possessed naked, monosporangiate flowers, since these characters also are common to most gymnosperms.

Before discussing this matter further, reference may be made to the possibility of there being two entirely separate lines of dicotyledons. Reasons have been given for believing that angiosperms are monophyletic. Now if the similarity between dicotyledons and monocotyledons is close enough to warrant such a conclusion, then, since the similarity is so much more striking among dicotyledons themselves, this conclusion seems all the more certain in the latter case. In the group Dicotyledoneae the diversity in the development and structure of the gametophytes and embryos is surely less marked than is the diversity among angiosperms as a whole, and so difficulties are increased accordingly if any other than a monophyletic theory is proposed for the phylogeny of dicotyledons. It is certainly true that the reproductive structures and organs of spermatophytes are among their least plastic features. While it is dangerous to emphasize too strongly the importance of even the most stable character to the exclusion of others, nevertheless, if the view that the embryo sac points unmistakably to a

monophyletic origin of angiosperms is incorrect, a more satisfactory one has never been advanced. The homoplastic explanation seems well-nigh inconceivable. Besides, in the morphological and anatomical structure of seedlings and mature plants all dicotyledons are essentially alike, so most of the important differences, as has been indicated above, are in the flowers. Taking all the evidence into account then, it seems likely that all dicotyledons are of one stock, and that the monocotyledons have arisen as one or more branches of this stock.

If then dicotyledons are monophyletic, which are the most primitive? If, as ARBER and PARKIN (2), WETTSTEIN (31), and others suppose, they have been derived from gymnosperms, from what particular gymnospermous stock may they have come? The Gnetales and more recently the extinct Bennettitales have each been thus designated. Even if dicotyledons originated from a gymnospermous stock, opinions will differ as to which is the parent group, depending on whether one regards naked, monosporangiate, anemophilous flowers, or entomophilous, amphisporangiate ones with a perianth as primitive.

The entomophilous habit seems clearly associated with much in the evolution of angiosperms, but it does not necessarily follow that primitive angiosperms were entomophilous. That anemophilous angiosperms may succeed and persist in competition with entomophilous forms is well illustrated by such groups as the Amentiferae and Gramineae.

Few, if any, believe that the flower of any existing angiosperm is like the primitive angiospermous flower. Whether this primitive flower was monosporangiate or amphisporangiate it does not follow, though this is quite possible, that any particular flower of today is the direct descendant of a similar type in its ancestor. It is certain that in many instances amphisporangiate flowers have become monosporangiate, and that perianths have been more or less completely lost.

That the opposite may have occurred, however, is less easily proven. ARBER and PARKIN (2), for example, object to ENGLER'S view (8) that the monosporangiate Apetalae are primitive among dicotyledons, by saying that "it must be assumed that the perianth

is evolved *de novo* and is an organ *sui generis*." But suppose we do assume that primitive angiosperms possessed a perianth, the origin of the perianth still remains to be explained. Even if we say that it is a direct derivative from a Bennettitean ancestor, its phylogenetic origin still remains a mystery. So if a perianth developed in some manner or other among the Bennettitales, might not the same thing have occurred among angiosperms, even long after they became a distinct group of plants?

The question whether primitive angiospermous flowers were monosporangiate or amphisporangiate presents even greater difficulties than that concerning the primitiveness of the perianth. If angiosperms have descended from gymnosperms we would rather expect primitive flowers to be monosporangiate. Evidence from gymnosperms that amphisporangiate flowers are primitive rests almost entirely on a single, extinct, much specialized group of plants, the Bennettitales. This group no doubt represents, as COULTER (7) suggests, "the end of a gymnosperm phylum." Moreover, that the proanthostrobilus of the Bennettitales corresponds closely to such a flower as that of *Magnolia* or *Liriodendron* still remains undecided. The resemblance is remarkable, yet if the view (17) that the Bennettitean inflorescence is a compound structure is correct, and if the flower of *Magnolia* is not compound, then the resemblance becomes only a superficial one.

If then we go back far enough in the evolution of angiosperms, the probability seems strong that the group was monosporangiate. Amphisporangiate flowers are unknown below angiosperms except in the Bennettitales and possibly *Welwitschia*. Although in a number of respects angiosperms and Gnetales have developed along parallel lines, it is now generally believed that the Gnetales are not transition forms leading to angiosperms. This view, however, does not preclude the possibility of common ancestry in the distant past. The Gnetales likely represent the end of a gymnospermous phylum just as the Bennettitales do. So neither group represents the direct progenitors of angiosperms.

COULTER and CHAMBERLAIN (7) say: "It is recognized that in the evolution of strobili among gymnosperms there were probably two distinct tendencies: a monosporangiate strobilus (Cyca-

dales, Cordaitales, Ginkgoales, Coniferales), and a bisporangiate strobilus with the anthostrobilus arrangement of sporophylls (Bennettitales, Gnetales, and leading to angiosperms)." This view apparently involves the idea that the Bennettitales, Gnetales, and angiosperms belong to one stock, but the relationship between the three groups at their origin may have been anything but close. Even though angiosperms are prevailingly amphisporangiate today this may not have been the condition among their remote ancestors; in fact it seems probable that those ancestors possessed monosporangiate flowers.

Though primitive angiosperms possessed monosporangiate, naked, anemophilous flowers, it is evident that they did not become the dominant group of plants until they developed amphisporangiate flowers with a perianth, and became entomophilous. This view is evidently opposed to the one that the present day angiosperms have been derived from the Bennettitean stock. The main reason for this belief is that except for resemblance in the fructifications, which may be quite superficial, the two groups are entirely different in structure. Indeed, had the Bennettitean proanthostrobilus never been discovered, probably no one would have ever suspected close relationship between such widely different groups. Leaving out of consideration the nature of the inflorescence, the following may be noted with reference to the Bennettitales: their seeds are of the gymnospermous type; the microsporophylls, microsporangia, and ramentum are fernlike in character; the external appearance and anatomy of the stem and leaf indicate relationship with cycads. All these characters suggest relationship with Cycadofilicales, while their strobili alone indicate a possible connection with angiosperms. It seems on the whole much simpler and safer to conclude that the Bennettitean proanthostrobilus and the angiospermous anthostrobilus are nothing more nor less than the results of homoplastic development, and that if they indicate relationship at all, it must be of the remotest kind, dating from a time prior to the origin of the Bennettitales as a separate stock, a time when neither true Bennettitales nor angiosperms had ever existed.

If then angiosperms were primitively anemophilous with naked

monosporangiate flowers, why are the present Monochlamydeae not to be regarded as the most primitive living members? This is because various features of the Monochlamydeae indicate reduction and not primitiveness. If, for example, the entire group as constituted by WETTSTEIN (31) be considered, it is found to be prevailingly syncarpous. This surely is not a primitive feature. Again, in various families there are closely related genera, even species, some of which possess a perianth, while in others it is rudimentary or entirely absent. It is difficult, in such cases at least, to conceive of the latter condition as primitive. Besides, the stamens and carpels may vary in number even in closely related genera and species. That the smaller numbers in such cases are derived seems to be a reasonable conclusion. Moreover, the inflorescences among the Amentiferae, for example, are compound structures exhibiting considerable complexity. This too can be more readily interpreted as derived and not primitive. Amphisporangiate flowers also occur in certain members of at least 7 families included by WETTSTEIN among the Monochlamydeae. Such cases add much weight to the view that the monosporangiate condition throughout the group is secondary and not primitive.

We may conclude then that considering existing angiosperms, the evidence at present available is in the main opposed to the view that they have been derived from forms at all closely related to the Bennettitales. The one striking similarity between modern angiosperms and the Bennettitales may well be a result of homoplasy. Among existing angiosperms, assuming that they are monophyletic, the derivation of forms having monosporangiate, naked flowers from those possessing amphisporangiate flowers, bearing an undifferentiated perianth, seems far simpler than the reverse. So, while agreeing with ARBER and PARKIN, HALLIER, and SENN in general with reference to those features which they believe are primitive among existing angiosperms, there seem to be no very adequate grounds for concluding that primitive angiosperms were provided with entomophilous, amphisporangiate flowers bearing a perianth. The opposite seems much more probable. If the latter is true, scarcely a suggestion of relation-

ship with the Bennettitales remains. But even if the former is true, it would seem hazardous to hold that angiosperms are more closely related to the Bennettitean stock than to any other gymnospermous stock. COULTER and CHAMBERLAIN (7) say that "the Cycadofilicales are so fernlike in every feature except their seeds, that their derivation from some ancient fern stock (called provisionally Primofilices) is as certain as phylogenetic connections can be. The origin of the Cordaitales therefore presents two alternatives: either they arose independently from the same ancient fern stock, or they were differentiated from the Cycadofilicales very early." The same two alternatives present themselves, it seems, in the case of angiosperms. Which view we accept is of little consequence since probably neither can be proven. Either view would make the connection between angiosperms and the Bennettitales, as we know them, a most distant one.

Let us now turn to our original question concerning the primitiveness of the Magnoliaceae among existing angiosperms. While the following list of characters of the Magnoliaceae is incomplete, it doubtless includes most of the more important ones that may be considered primitive: (1) the ordinary 8-nucleate type of embryo sac; (2) dicotyledony; (3) undifferentiated perianth; (4) amphisporangiate flower; (5) entomophily; (6) elongated conical floral axis; (7) actinomorphy; (8) indefinite number of free floral organs arranged spirally; (9) hypogyny; (10) apocarpy; (11) woody stems; (12) occasional absence of tracheae in the vascular bundles.

It is quite generally agreed that the last 7 of these characters are relatively primitive wherever found among angiosperms, or if not that they are of minor importance as evidence of phylogeny. The first two characters, since they are common to nearly all dicotyledons, are valueless as criteria for determining primitiveness. There are left three characters which may be either primitive or derived, namely, undifferentiated perianth, amphisporangiate flowers, entomophily. These three characters have no doubt developed together and are closely bound up with the evolution of angiosperms. If not, then naked, monosporangiate, anemophilous flowers must indicate primitiveness where found in existing

forms. We believe, for reasons previously given, that the type of flower found among the Magnoliaceae is primitive.

The present investigation adds little toward the solution of the plexus of problems involved in the origin of angiosperms and the relative primitiveness of existing groups. An opportunity was offered, however, to determine whether such a study as the present one of a possibly primitive group of angiosperms might yield any results of either positive or negative value as a contribution to present theories; to suggest new points of view and especially to emphasize certain old ones; to review briefly some of the principal theories of the present day on the primitiveness and origin of monocotyledons and dicotyledons; and finally to criticize where it seemed this might be helpful in bringing about in the future reinforcement or correction either of earlier theories or of the views expressed in this paper.

Summary

1. In both *Magnolia* and *Liriodendron* the sporogenous tissue in the anther is differentiated early in the winter. Tetrads develop by the simultaneous method and the pollen grains when mature are binucleate in *Magnolia*, two-celled in *Liriodendron*. The tapetum originates from the sporogenous tissue.

2. The x number of chromosomes in each species is 19.

3. The ovules in both species are marginal, anatropous, and provided with two integuments. The megaspore mother cell in each species, by two successive divisions, produces 4 megaspores, of which the innermost is functional. The mature embryo sacs are of the ordinary 8-nucleate type and fertilization probably occurs as usual.

4. The endosperm of *Magnolia* is cellular from the beginning of its formation and is abundant in the mature seed, surrounding a small, typically dicotyledonous embryo. The first division in the development of the embryo is transverse, the second longitudinal to the long axis of the embryo sac. The embryo has a well defined suspensor and no evidence of monocotyledony was found.

5. The seed of *Magnolia* possesses three coats: an outer fleshy

and within this a stony one, both developed from the outer integument, and a thin inner one from the inner integument.

6. The flowers of both species differ from the euanthostrobilus of ARBER and PARKIN only in having anatropous ovules and two-seeded carpels.

7. In *Liriodendron* the seedling possesses in the cotyledons and upper part of the hypocotyl the structure generally found associated with tetrarch roots, but the root is diarch.

8. All Magnoliaceae possess certain common anatomical characters, while others are peculiar to particular groups. The bundles in the petioles and peduncles of *Liriodendron* and various species of *Magnolia* are somewhat scattered, a feature the interpretations of which vary.

9. PORSCH's archegonium theory gives no suggestion as to what type of angiospermous embryo sac is primitive.

10. A consideration of all available evidence in the light of present theories very strongly favors the conclusion that the ordinary 8-nucleate type of angiospermous embryo sac is the most primitive. But in view of its wide distribution among all groups of angiosperms, its occurrence either among the Magnoliaceae or in any other family is no evidence of the primitiveness of that family.

11. Angiosperms are believed to be monophyletic, especially on account of the uniformity in the development and mature condition of the gametophytes, and because of similarity in seedling structure of monocotyledons and dicotyledons.

12. The view that dicotyledons have been derived from monocotyledons, as advanced by WORSDELL, rests too largely on assumption.

13. The theory that monocotyledons originated from dicotyledons is supported by evidence from gametophytic development and anatomical structure of seedlings and mature plants, as well as by indications that primitive angiosperms possessed a cambium and were dicotyledonous, and by the conclusion that the peculiarities of anomalous dicotyledons are secondary. The important differences between monocotyledons and anomalous dicotyledons on the one hand, and ordinary dicotyledons on the other, are believed

by many to be due to response to the peculiar environmental conditions surrounding the former.

14. Theories differ especially as to whether amphisporangiate, entomophilous flowers bearing a perianth, or naked, anemophilous, monosporangiate ones are primitive among dicotyledons. The writer holds, for reasons given, that in the remote past the ancestors of the dicotyledons (and so of all angiosperms) possessed naked, unisexual flowers, but that among existing groups hermaphrodite flowers provided with a perianth are primitive, and that the naked, unisexual forms existing today have been secondarily derived from the latter; moreover, that the appearance of entomophily, the amphisporangiate condition, and the perianth have been very important features in the evolution of modern angiosperms.

15. The Bennettitales, Gnetales, and angiosperms may have had common ancestors if we go back to a time prior to that when the Bennettitales became a distinct line. It seems reasonable to conclude either that angiosperms were derived from the same ancient fern stock from which the Cycadofilicales originated, or else that they were differentiated from the Cycadofilicales at a very early time.

16. In conclusion, it is believed that the most primitive of existing angiosperms are to be found among the Magnoliaceae or related forms, and not among forms with naked, monosporangiate, anemophilous flowers.

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EXPLANATION OF PLATES I–III

All drawings were made with the aid of a camera lucida from microtome sections.

Abbreviations used: *a*, antipodal; *ar*, archesporium; *cb*, cortical bundle; *cc*, central cylinder; *c*, cotyledon; *e*, egg; *em*, embryo; *es*, endosperm; *fvb*, fibrovascular bundle; *ii*, inner integument; *m*, mechanical tissue; *mgmc*, megaspore mother cell; *mg*, megaspore; *mcmc*, microspore mother cell; *mp*, micropyle; *n*, nucellus; *oi*, outer integument; *p*, pith; *pc*, parietal cells; *ph*, phloem; *pn*, polar nucleus; *pt*, pollen tube; *st*, sporogenous tissue; *su*, suspensor; *sy*, synergid; *t*, tapetum; *tc*, tapetal cell; *te*, tetrad; *v*, vacuole; *x*, xylem.

Liriodendron

FIG. 1.—Part of longitudinal section of microsporangium and wall; wall somewhat diagrammatic; ×350.

FIG. 2.—Pollen mother cells dividing; ×350.

FIG. 3.—Section of pollen grain with reduced number of chromosomes; ×600.

FIG. 4.—Section of two-celled pollen grain; ×350.

FIG. 5.—Longitudinal section of young ovule; ×300.

FIG. 6.—Megaspore mother cell divided; ×350.

FIG. 7.—Longitudinal section of ovary showing tetrad of megaspores deeply buried within nucellus; ×350.

FIG. 8.—Longitudinal section of micropylar end of embryo sac; ×350.

FIG. 9.—Polar nuclei fusing; ×350.

FIG. 10.—Longitudinal section of antipodal end of embryo sac; ×350.

FIG. 11.—Transverse section of petiole showing arrangement of fibrovascular bundles; ×50.

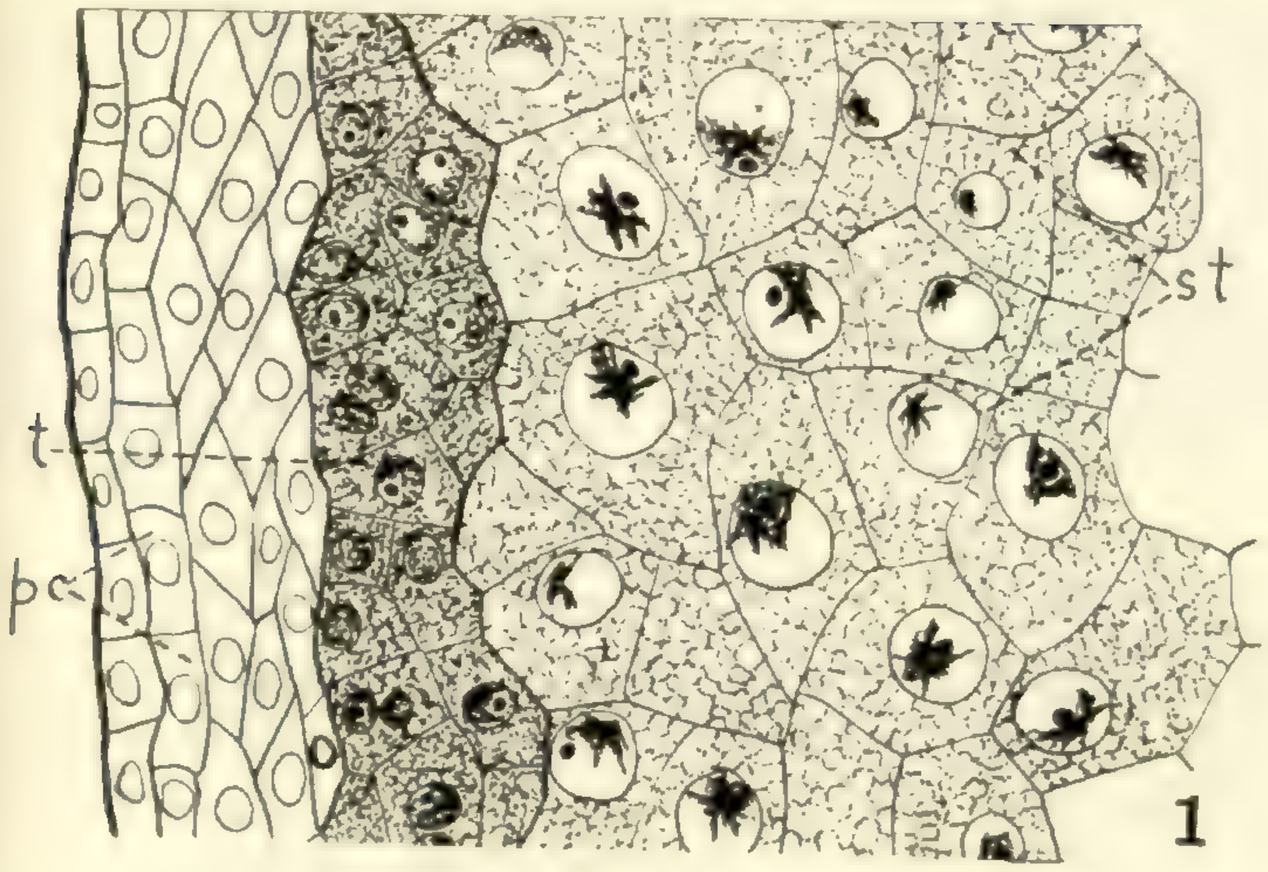
FIG. 12.—Transverse section of portion of central region of diarch root; ×590.

FIG. 13.—Transverse section of hypocotyl; ×50.

FIG. 14.—Transverse section showing details of the portion of fig. 13 indicated by line *ab;* ×300.

Magnolia

FIG. 15.—Longitudinal section of part of anther; sporogenous tissue differentiated; ×350.



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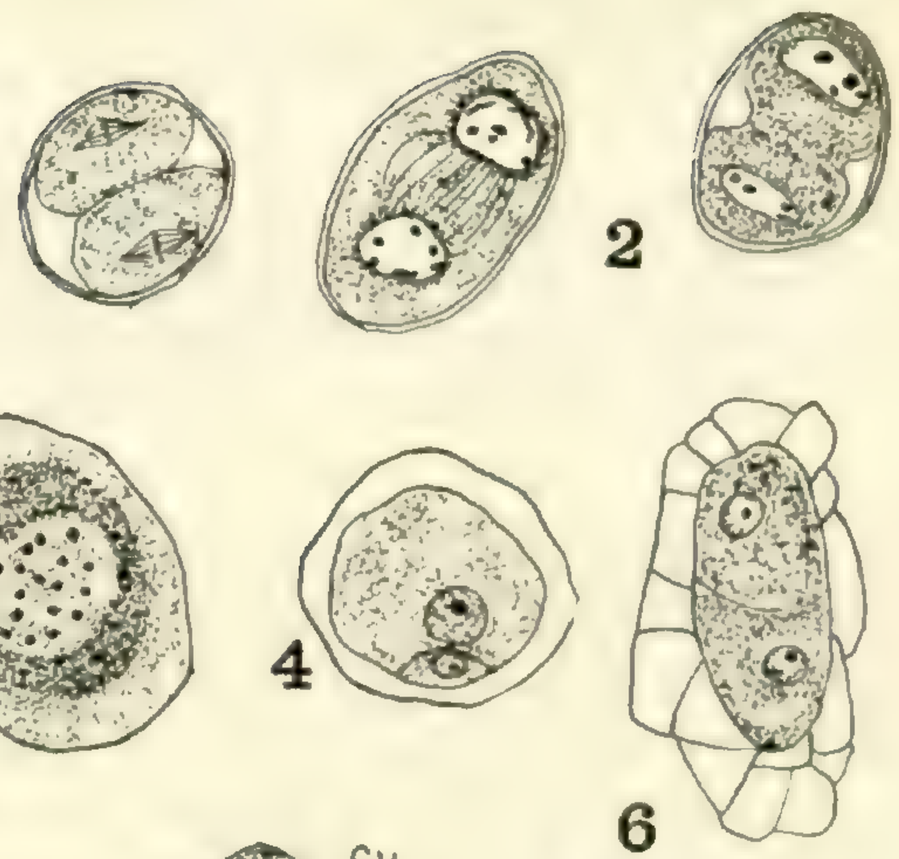

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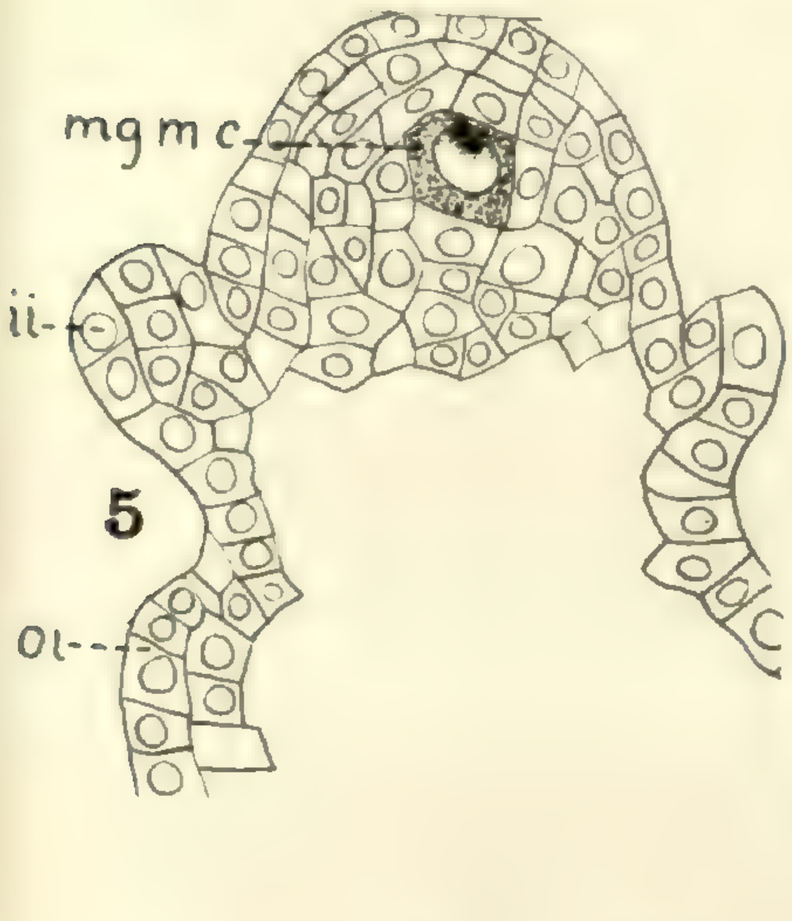

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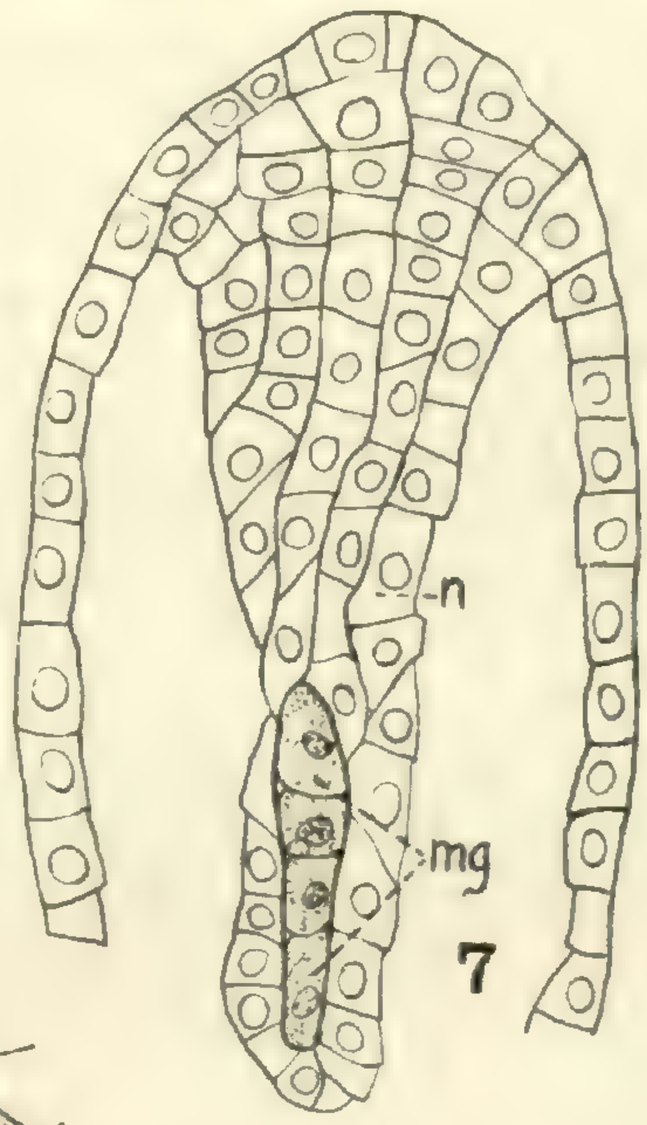

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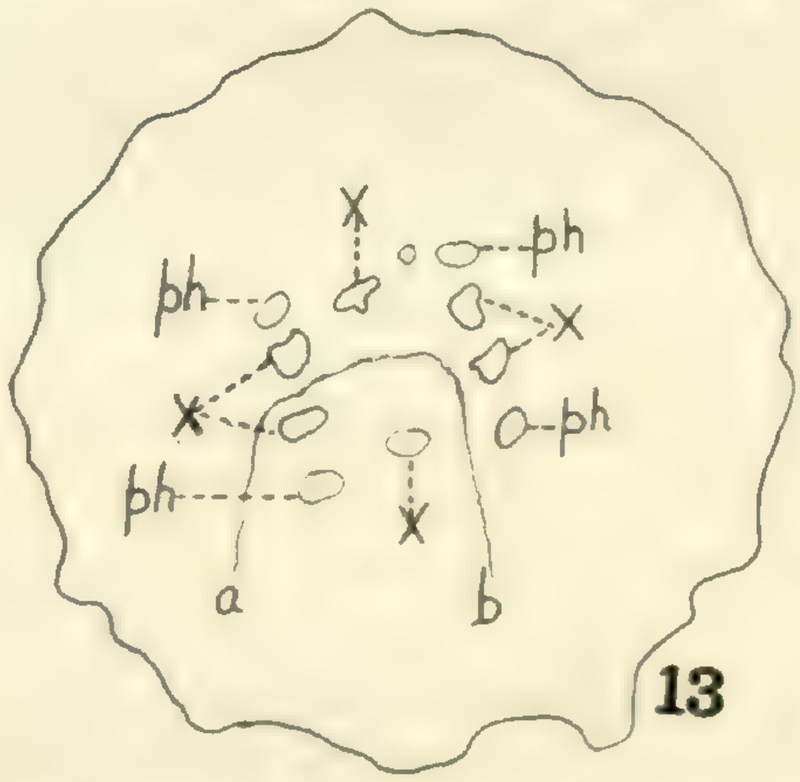

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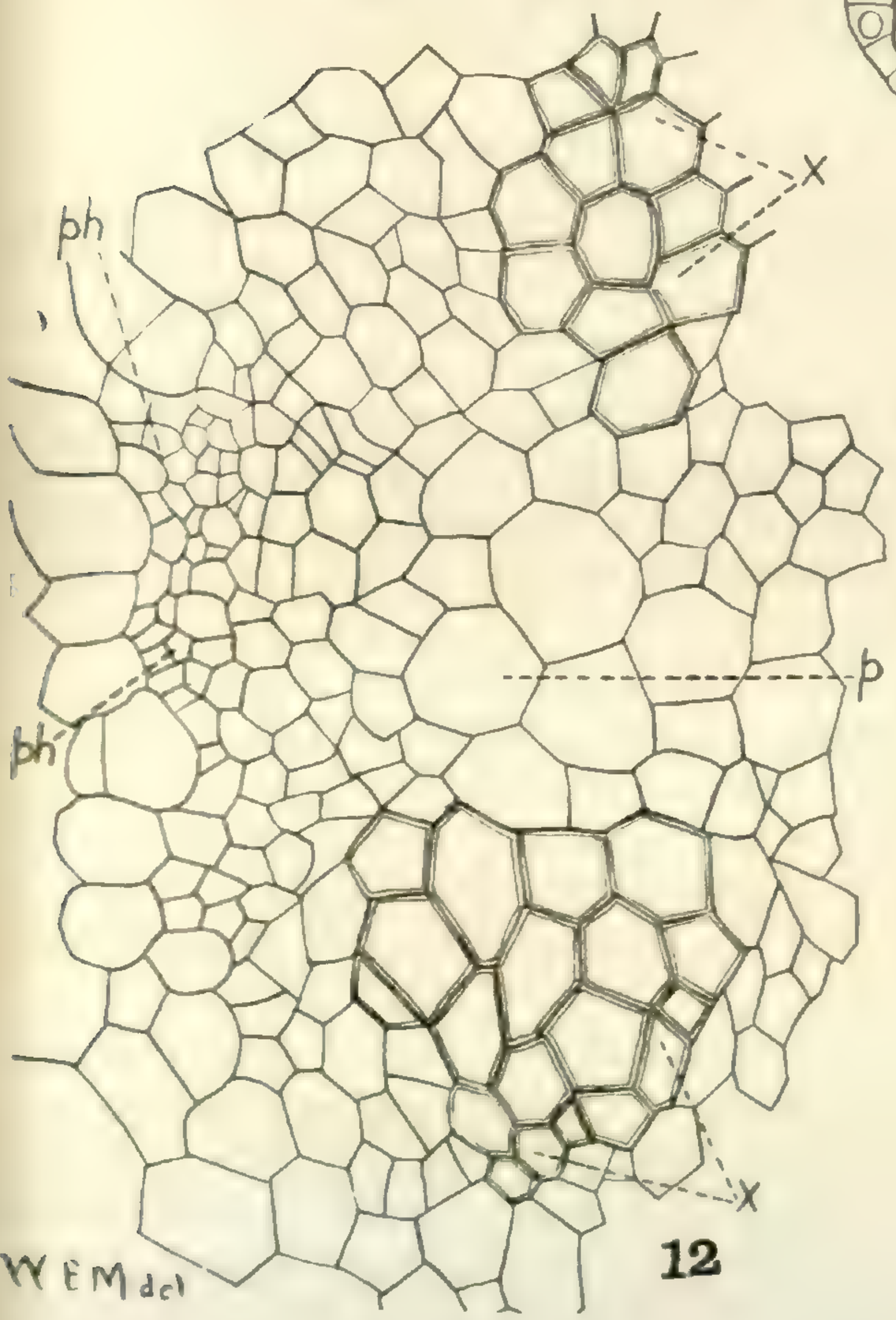

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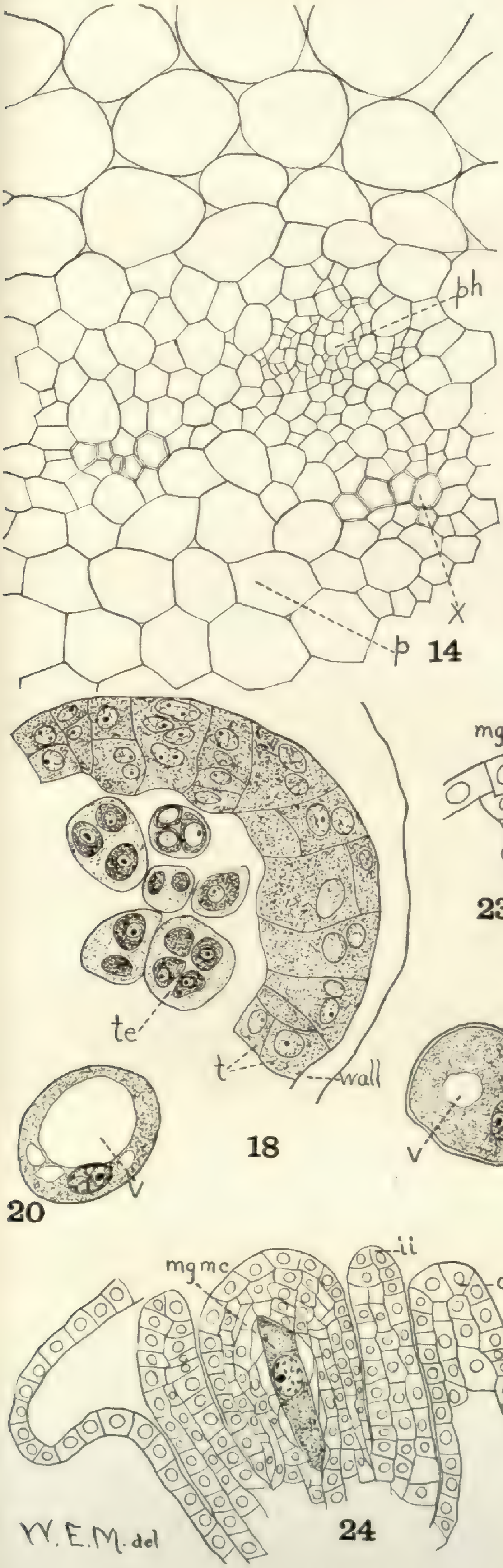
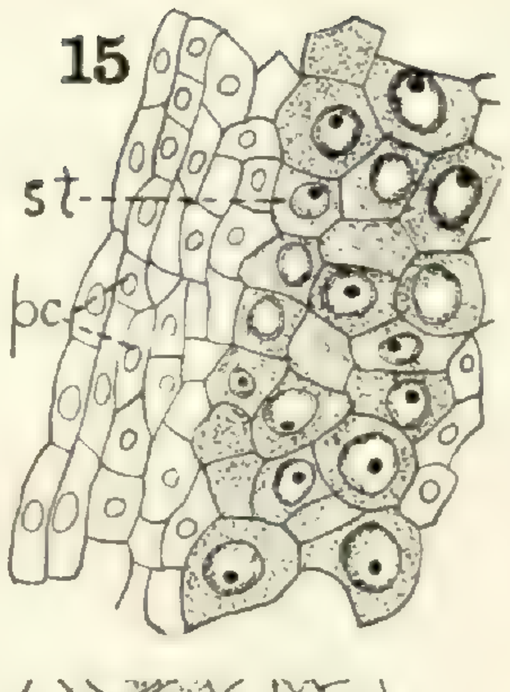
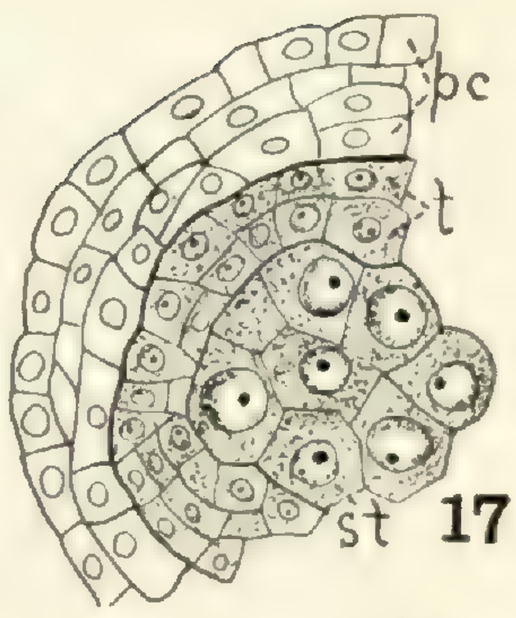
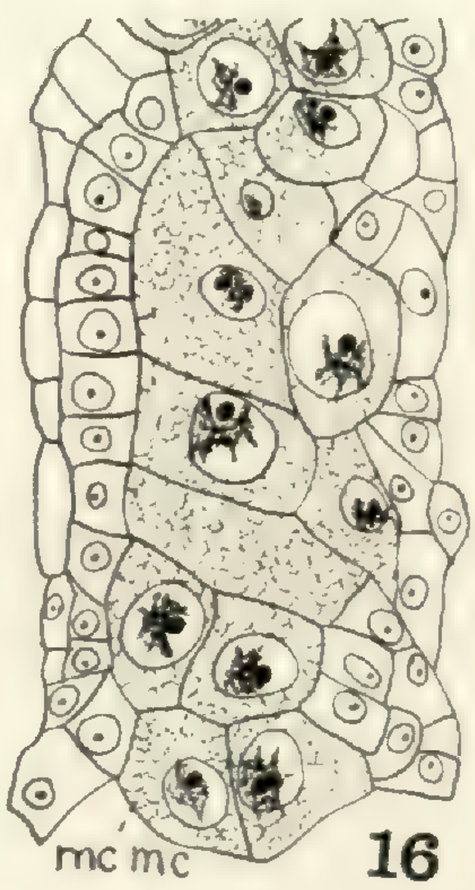
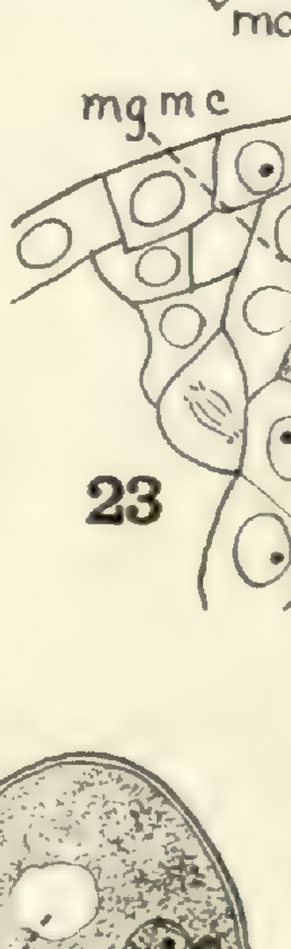
MANEVAL on MAGNOLIACEAE

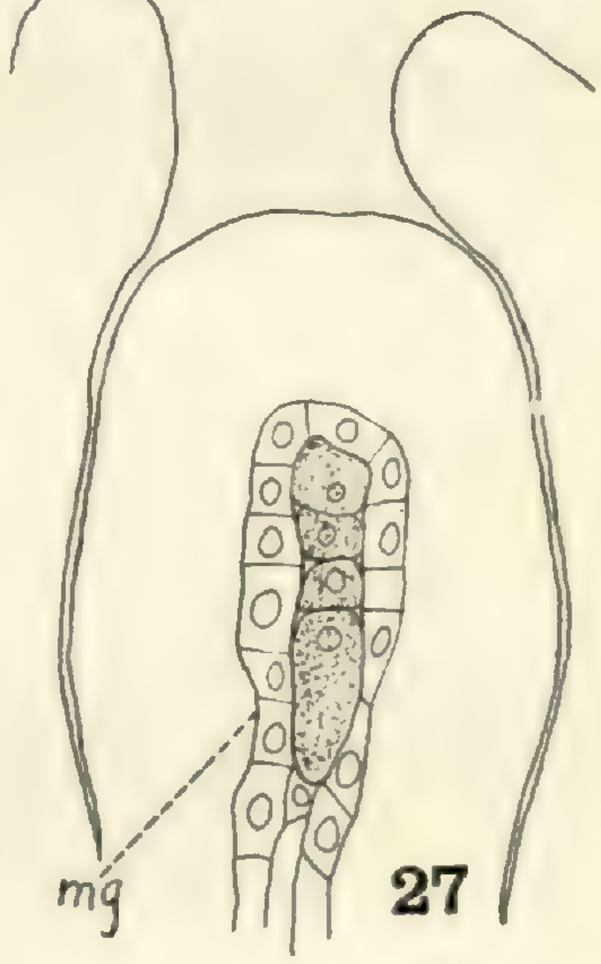


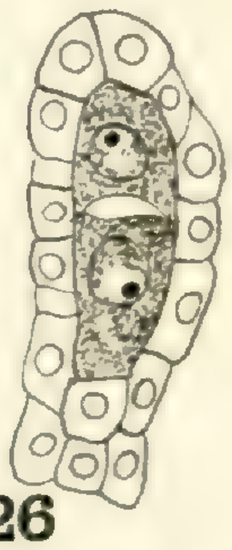


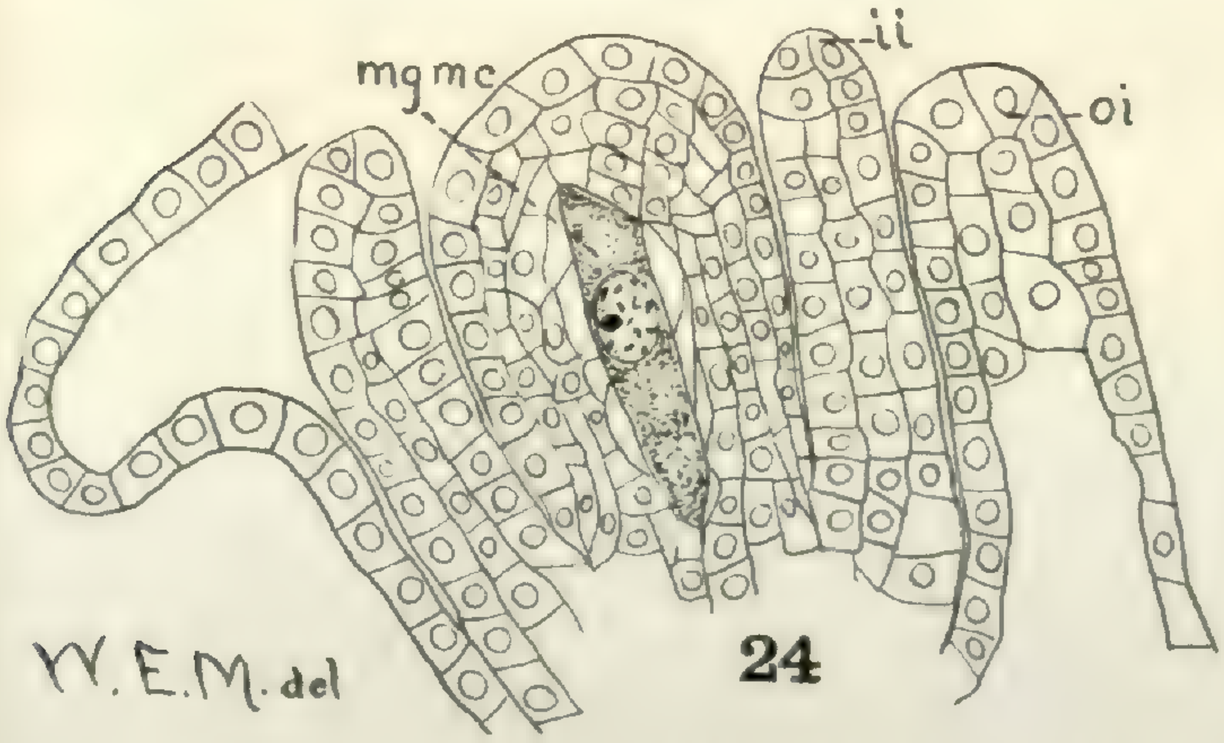












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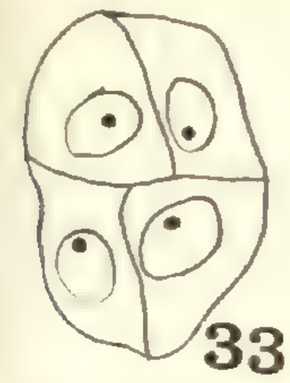
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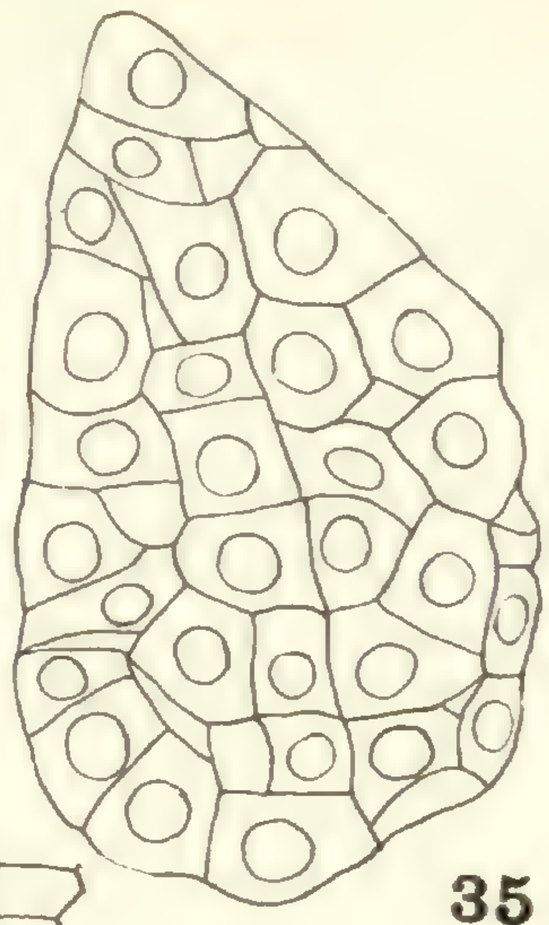
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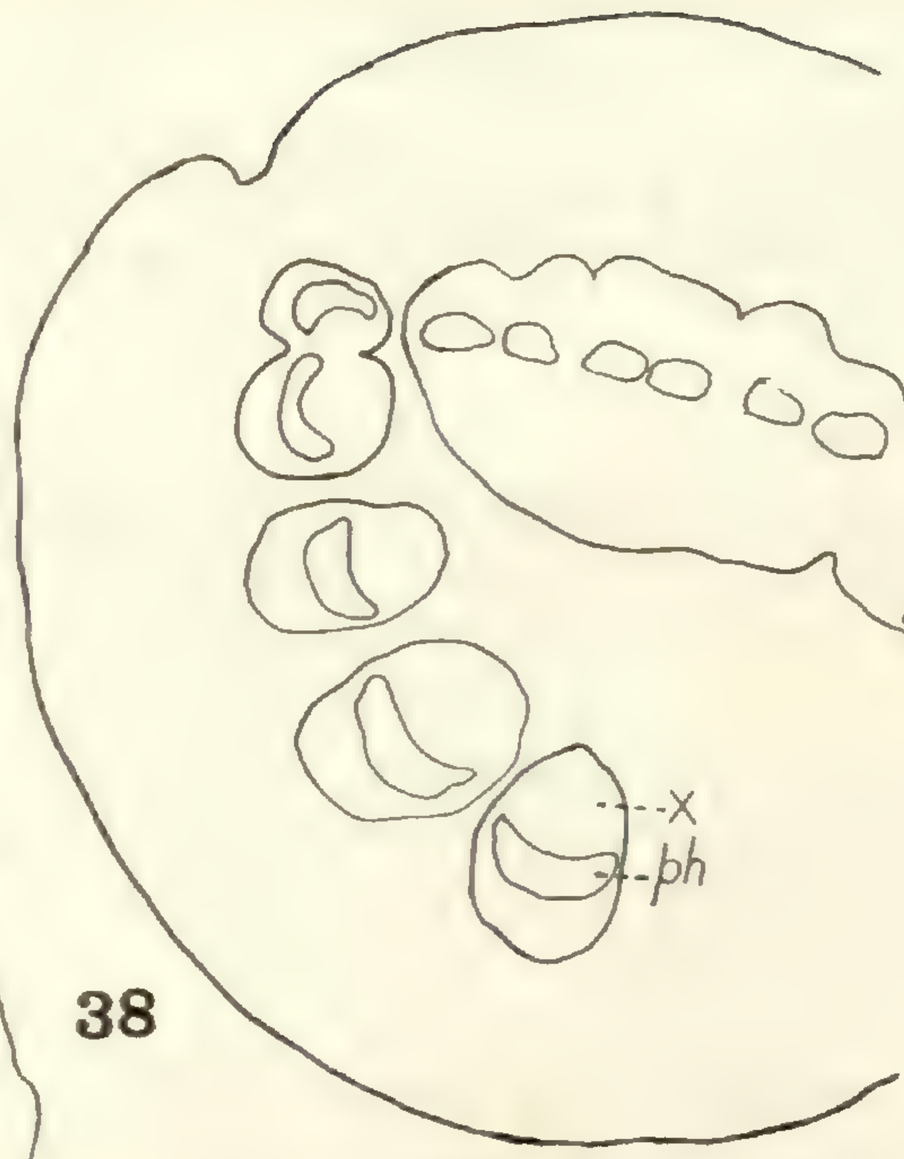
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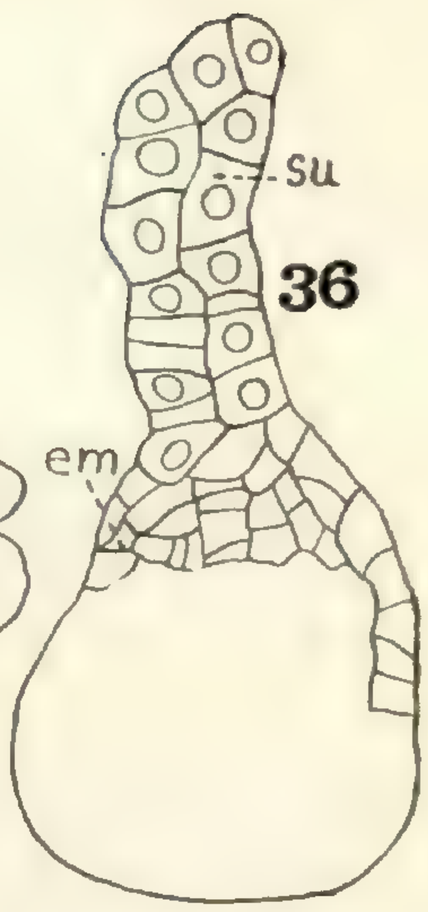
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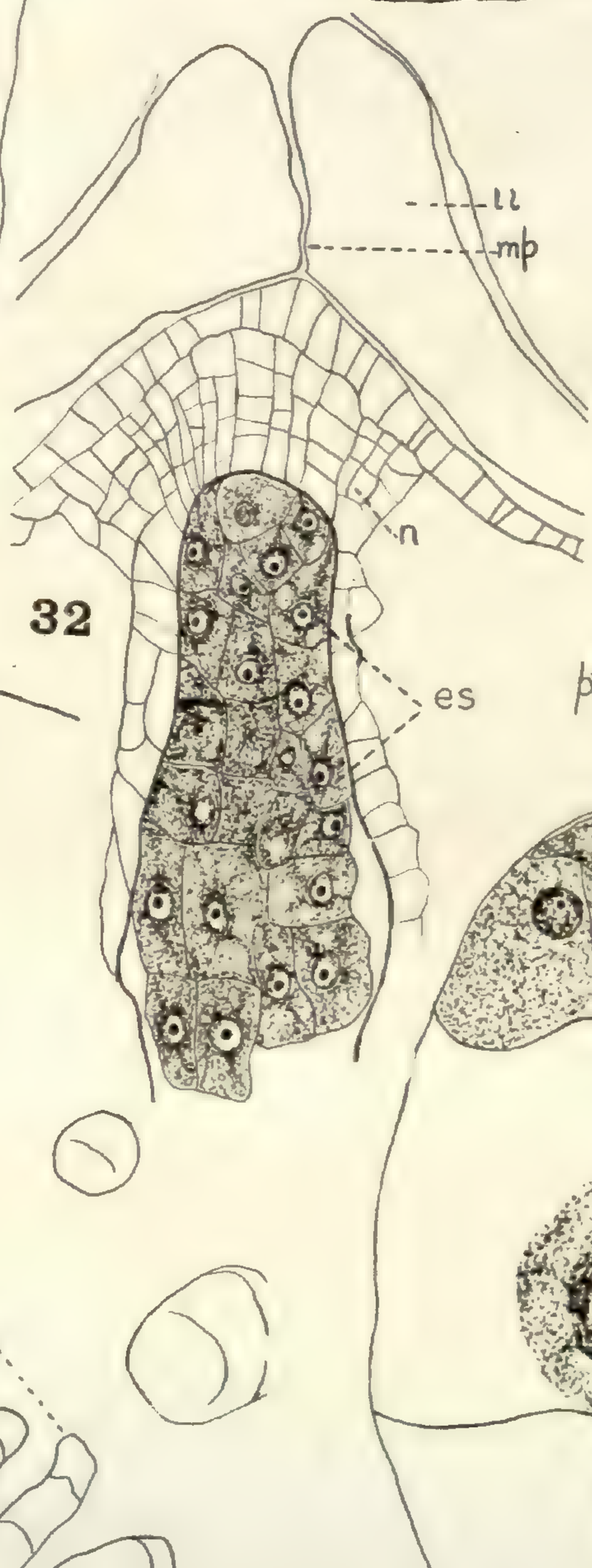
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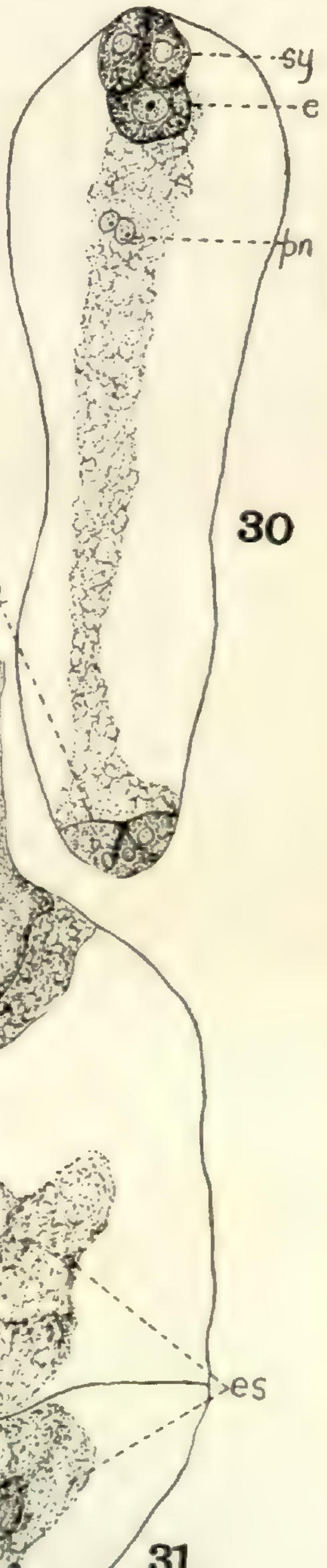
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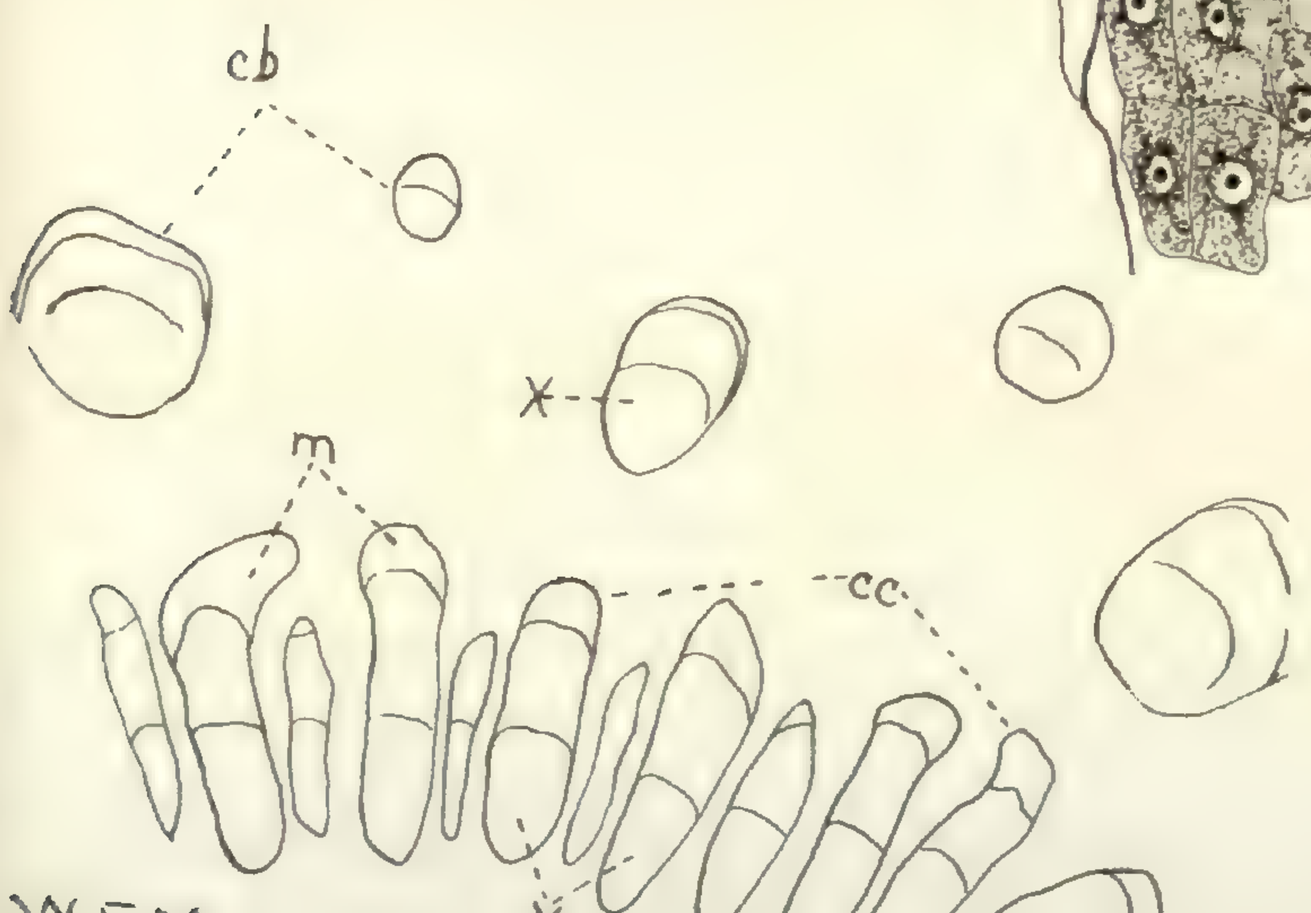
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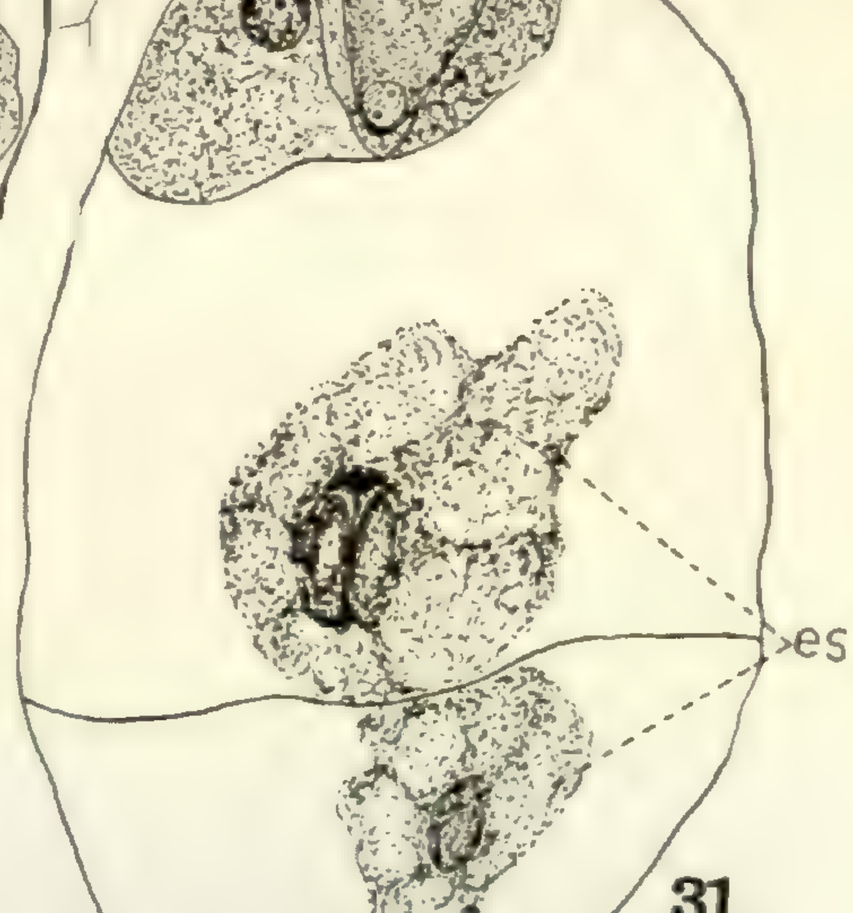
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W.E.M. del.

MANEVAL on MAGNOLIACEAE

FIG. 16.—Longitudinal section of part of anther; microspore mother cells in synapsis; ×300.

FIG. 17.—Transverse section of anther; tapetum and sporogenous tissue differentiated; ×350.

FIG. 18.—Transverse section of anther; tetrads of microspores; ×300.

FIG. 19.—Section of pollen grain with reduced number of chromosomes; ×500.

FIG. 20.—Uninucleate pollen grain; ×350.

FIG. 21.—Binucleate pollen grain; ×350.

FIG. 22.—Longitudinal section of ovule with archesporial cell; ×300.

FIG. 23.—Longitudinal section of ovule through megaspore mother cell and tapetal cell; ×500.

FIG. 24.—Longitudinal section of ovule; megaspore mother cell; integuments; ×300.

FIG. 25.—First division of megaspore mother cell; ×350.

FIG. 26.—Megaspore mother cell divided; ×350.

FIG. 27.—Tetrad of megaspores; ×350.

FIG. 28.—Longitudinal section through binucleate embryo sac; ×350.

FIG. 29.—Longitudinal section through tetranucleate embryo sac; ×350.

FIG. 30.—Longitudinal section through mature embryo sac; ×350.

FIG. 31.—Micropylar end of embryo sac; pollen tube; two-celled endosperm; ×600.

FIG. 32.—Longitudinal section of micropylar end of embryo sac showing early condition of endosperm; ×300.

FIG. 33.—Section of four-celled embryo; ×500.

FIG. 34.—Section of eight-celled embryo; ×500.

FIG. 35.—Longitudinal section through an older embryo; ×500.

FIG. 36.—Longitudinal section of embryo and suspensor; ×300.

FIG. 37.—Longitudinal section of embryo from mature seed; ×50.

FIG. 38.—Transverse section of petiole; ×50.

FIG. 39.—Transverse section of portion of a fibrovascular bundle of a petiole; ×300.

FIG. 40.—Transverse section of portion of peduncle; ×50.

THE MATURATION PHASES IN SMILAX HERBACEA

MARION G. ELKINS

(WITH PLATES IV–VI)

The material for this paper was collected in the spring of 1909 in the vicinity of New Haven, Connecticut. Both staminate and pistillate flowers were obtained with a view to studying nuclear conditions in both sexes. Staminate flowers, gathered on May 14, supplied nearly all the stages desired, as the flower buds in each inflorescence exhibited varying degrees of development. The pistillate flowers, maturing more slowly, were fixed May 26 and June 2. The series obtained from this material was very incomplete, only a few stages of the prophase of the heterotypic division being procured. Traces of the megaspores were visible in some flowers, but in most cases their location was represented by a dark, irregular line which suggested crushing or imperfect fixation.

Various killing fluids were used, but only two proved of any value, namely Flemming's fluid (weaker solution) and Juel's fluid. Sections were cut 6μ in thickness and stained with Flemming's triple stain or Haidenhain's iron haematoxylin.

The maturation phases in the microsporangium

The earliest observations of the sporogenous tissue were made after the telophase of the last vegetative mitosis and before the differentiation of the tapetum. Excluding the outer layer of cells in this tissue, which eventually become tapetal, the remaining cells are virtually pollen mother cells, and after a slight increase in size are ready for the phenomena characterizing meiosis. The nuclei of the young spore mother cells show small chromatin bodies or granules of variable size scattered through the finely granular linin meshes. A distinct reticulum is not present. Often the chromatin bodies may be seen in pairs or groups of four, but their distribution is generally irregular and does not warrant a conclusion that pairing is their typical arrangement.

A multinucleolate condition is typical of the nuclei of these cells. The nucleoli are variable in number and size and often somewhat angular in outline; several small bodies appear attached to the nucleoli (figs. 2, 3), which resemble papillae and will be so designated during the following description. As late as diakinesis nucleoli have been observed with one or more of these papillae. In the material prepared with the triple stain the nucleoli of the heterotypic prophase show one or more glistening white spots; these were at first considered to be vacuoles, but there is also the possibility that they represent papillae viewed on the upper surface of the nucleoli. The microspore mother cell in the early prophase is sometimes uninucleolate, though more often provided with two large nucleoli. However, at synapsis there is never more than one large nucleolus, which is no longer angular and is usually provided with a single papilla.

In connection with the study of the microspore mother cells of the early prophase, observations were made on the somatic nuclei of the nucellus. The appearance of the chromatin bodies and the nucleoli in such a nucleus (fig. 27) agrees very closely with that given for the nuclei of the young spore mother cells.

The author believes the uninucleolate condition (fig. 5) to be the result of union of the nucleolar elements. Fig. 1 shows two nucleoli connected by a short, deeply stained strand, while fig. 2 shows two nucleoli in a later stage of fusion. The papillae described above are probably nucleolar fragments or very small nucleoli which are in the process of fusing with the larger nucleoli. Fusion of all the nucleolar matter apparently does not take place; as late as diakinesis small globular bodies are often found which are distinct from both the nucleolus and the chromosomes. The papillate condition of the nucleolus also persists until the nucleolus disappears at the metaphase.

GATES (14) describes parallel phenomena in the sporogenous cells of *Oenothera rubrinervis*. Here the nucleus is provided with one large nucleolus accompanied almost invariably by smaller nucleolar bodies. Fusion of these bodies takes place; the number present in later stages depends on the amount of fusion. This seems to vary. One large nucleolus is always present until the

disappearance of the nuclear membrane; there are also one or two small nucleolar bodies which remain through the metaphase of the heterotypic division and are sometimes observed on the homotypic spindle.

This papillate appearance of the nucleolus may be interpreted in quite another way, namely, as a process of chromatin budding from the nucleolus. CARDIFF (5) figures nucleoli with similar papillae in young spore mother cells, and suggests that a papilla may represent the beginning of a chromatin thread formed from the nucleolus. Miss NICHOLS (32), in a study of several species of *Sarracenia*, concludes that the nucleolus of the pollen mother cells elaborates the chromatin, and figures nucleoli with small bodies attached, which represent the chromatin emerging from the nucleolus. DARLING (7) describes the budding of chromosomes from the nucleolus in the prophase of the heterotypic division in *Acer Negundo*. In the somatic nuclei of the root tip of *Phaseolus*, WAGER (43) describes the nucleolus as being connected with suspending fibers along which the chromatin from the nucleolus passes. These fibers become much thickened with the accumulation of chromatin and finally break up into chromosomes. With the loss of chromatin the nucleolus shrinks, becomes detached from the chromosomes at the metaphase, and finally disappears. The conclusions of WAGER, however, are disputed by MARTINS MANO (26), who made a similar study of *Phaseolus* and concluded that the chromosomes are not of nucleolar origin. He advances this interpretation: at the telophase certain portions of the chromosomes are drawn out into threads which anastomose and form a chromatic reticulum. In the following prophase the reticulum gradually assumes the form of bands with connecting fibers; the bands contract and ultimately break up into chromosomes. The appearance that WAGER describes as "suspending fibers" MARTINS MANO interprets in another way. The nucleolus is formed independently from nucleolar substances, but in close contact with chromatin elements. When the perinucleolar vacuole is formed, there is a consequent repulsion of the nuclear reticulum; certain parts of it remain attached to the nucleolus by reason of the elasticity and viscosity of the chromosomes. If the nucleolus furnishes

any substance to the chromosomes it is not done by means of "suspending fibers."

Fig. 3 seems to show a condition like that described by DARLING and WAGER. There is a great similarity between the papillae attached to the nucleolus and the chromatin bodies lying free in the linin network. On the other hand, the presence of numerous darkly staining granules in the sporogenous and somatic nuclei and the presence of a nucleolus as late as diakinesis argue against the resolution of the nucleolus into chromatin bodies.

SYNAPSIS.—The term "synapsis" has been defined in two ways. Most botanists use the word to denote the period of maximum contraction of the chromatic elements in the prophase of the heterotypic division. Zoologists call this stage "synizesis" (McCLUNG **25,** JORDAN **20**) and assign the name "synapsis" to the period of approximation of parental chromosomes. GRÉGOIRE (**17**) defines synapsis as covering stages from leptonema to strepsinema. In this paper "synapsis" will be considered as synonymous with "synizesis."

The presynaptic phases in *Smilax herbacea* are apparently simple; the linin mesh contracts (fig. 6), drawing the chromatin bodies together into an increasingly close proximity. As there is no chromatic reticulum or pairing of thin filaments, the process resolves into the mutual approach of chromatic bodies. MIYAKE'S (**28**) description of chromatin behavior in *Lilium* corresponds closely to this account. During synapsis (fig. 7) the nucleolus is almost invariably at one side and projecting from the synaptic mass; delicate linin threads bridge the karyolymph and connect the chromatic ball with the nuclear membrane. For a time the granular nature of the chromatin is maintained; toward the end of synapsis, however, the chromatin becomes arranged in a much interwoven beaded filament (fig. 8).

Views in regard to synapsis and its importance range from those that discard it as of no significance or due to faulty technique (SCHAFFNER **37**) to those that assign to it the function of bringing together parental elements in closest union and mutual influence. LAWSON (**22**) denies the presence of any real contraction and states that the phase known as synapsis is due to an enlargement of the

nuclear cavity, the chromatic mass remaining the same size. In 1899 GUIGNARD (19) reported the absence of synapsis in *Naias major*. CARDIFF (5) has described this phase as the deferred culmination of fertilization. The later tendency is to admit its presence as a normal stage and to consider it only a time of great shortening and thickening of chromatic filaments (GRÉGOIRE 17, BERGHS 2, DAVIS 8). GRÉGOIRE in his discussion of synapsis admits that it is not a universal phenomenon. When it does occur, he believes it can have no rôle to play in the process of reduction, but is itself a result of certain nuclear activities. He further suggests that the appearance of synapsis may be emphasized by the growth of the nuclear cavity or by an artificial contraction caused by fixing reagents enhancing the natural contraction. GATES (16) states that it is evident many changes take place during synapsis, though there may be no interchange or influence between homologous chromosomes. He points out (15) that such influences may take place at any time during the sporophytic phase of the life cycle. ERNST (12) considers synapsis normal, otherwise a similar sensitiveness to fixing fluids ought to show in vegetative mitosis of corresponding stages. There is one possibility favoring artifact, namely, that the progressive stages of mitosis may be accompanied by chemical changes in the chromatic substance which cause different reactions to fixing fluids. It is difficult, however, to conceive of a chemical change occurring in a heterotypic prophase which would not also occur in a somatic prophase. Moreover, there is no experimental basis for this view, though NĚMEC (30) by microchemical tests demonstrated differences in the chromatin of resting and dividing nuclei.

The contraction of the nuclear contents is very striking in *Smilax herbacea* (cf. figs. 5, 6, 7), and is without doubt a normal condition. It is difficult to assign synapsis a rôle in *Smilax*. It is evident that the appearance of the nucleus after synapsis differs markedly from the preceding conditions; the chromatin elements pass into synapsis as distinct bodies and emerge in a homogeneous filament. Until more light is thrown upon this phase, we can only vaguely state that synapsis may facilitate the proper placing of the paired parental elements in the chromosomes and the chromo-

somes in the spireme. The chromosomes in *Smilax herbacea* never appear as definite units until the segmentation of the spireme.

POSTSYNAPSIS.—The much coiled filament of late synapsis emerges as a fairly thick thread slightly beaded (fig. 8), which later assumes a homogeneous character. The double nature of the spireme is early discernible (fig. 9). With the continued loosening of the knot the split is sometimes obliterated, but the spireme as it is distributed throughout the nuclear cavity is distinctly double; this separation of previously paired[1] chromatic elements does not appear simultaneously in all parts of the spireme (figs. 10, 11, 12). Shortening and thickening of the spireme proceeds as usual, followed by a sort of semi-segmentation of the double filament. At intervals along the spireme occur places where each longitudinal half is apparently constricted to a delicate thread (fig. 13). This appearance is due doubtless to incomplete transverse segmentation with subsequent pulling apart of the double segments; the attached portions thus are drawn out into fine threads. The bivalent chromosomes resulting from the completion of the transverse division of the spireme are long and slightly twisted about each other (figs. 14, 14*a*). Though not a typical strepsinema, this condition corresponds to strepsinema as described by GRÉGOIRE (17).

The shortening and thickening process continues, resulting in the characteristic diakinetic gemini, the univalent halves of which lie, indiscriminately (fig. 15), parallel, at right angles to each other, or in the form of V's or X's. Traces of linin threads can be seen attached to the ends of the chromosomes. The nucleolus is present at this stage (though not figured), but disappears before the metaphase. Small globular bodies scattered about among the gemini appear in many of the nuclei in addition to the nucleolus. These are probably small nucleolar bodies as previously described.

METAPHASE.—Many of the gemini retain the semblance of a V on the spindle (fig. 16), though occasionally the homologous chromosomes are oriented in a straight line. Intermediate stages occur between these two types of orientation. In many cases the chromosomes show a distinct splitting while at the equator; this is more marked as the chromosomes pass toward the poles, though

[1] Previous pairing is assumed to have taken place.

it does not become a complete fission (fig. 19). This is without doubt a genuine splitting preparatory for the homotypic mitosis.

As the first division is merely a separation of chromosomes, the true mitosis being deferred until the second division, it is not surprising that the fully formed chromosomes exhibit a tendency to fission, the normal consequence of a mature condition in meristematic cells, long before actual mitosis is permitted to take place. FARMER (13) expressed a similar idea when he said "with the inception of karyokinetic activity the spireme thread undergoes the longitudinal fission characteristic of ordinary somatic division, although the actual separation of these longitudinal halves is deferred until the next mitosis."

The separation of the chromosomes at the equator and their passage to the poles takes place in the usual manner. Frequently the chromosomes of one or more pairs separate and move away from the equator earlier than the majority (figs. 17, 18).

INTERKINESIS.—At the telophase the chromatic elements appear in the form of a spireme which is disposed about the periphery of the newly formed nuclear membrane. The daughter nuclei are usually elliptical, though sometimes they are slightly curved, presenting a concave surface toward the equatorial plate. MIYAKE (28) finds in *Lilium Martagon* a partial formation of a thread, but usually there is little change in the form of the chromosomes during interkinesis. In *Smilax herbacea* the split which was observed in the metaphase and anaphase, homotypic in nature, is sometimes faintly discernible, but usually lost to view. Vacuolation of the chromatin band, if it may be said to occur at all, is very slight. In fact, the transitory character of this phase does not call for extensive alveolization. We have here in reality a prophase of the homotypic division. GRÉGOIRE (17) describes the heterotypic division as a process intercalated in the prophase of the homotypic division.

Conditions reported during interkinesis vary in different plants. In *Oenothera gigas*, according to DAVIS (9), the daughter chromosomes maintain their form and distinctly show the homotypic split, thus giving an appearance similar to diakinesis. GATES (16), describing the same species, states that some of the chromosomes

pass through interkinesis in a compact condition, while others become vacuolate. In *Nephrodium molle* (YAMANOUCHI **45**) the chromosomes become vacuolate, but their identity is not lost. ALLEN (**1**) cites the formation of a spireme during the telophase of *Lilium canadense*. In *Pinus* and *Thuja* (LEWIS **24**) the identity of the chromosome is completely lost. NICHOLS (**31**) reports a similar condition in *Juniperus*.

HOMOTYPIC DIVISION.—In preparation for the second division the nuclear membrane disappears[2] and is succeeded by the formation of a spindle whose axis corresponds with the greater axis of the daughter nucleus. The daughter spireme is at first spread out on the spindle from pole to pole (fig. 21); later the chromatic mass contracts (fig. 22) and occupies a position at the equator of the spindle. At this time the spireme seems to be resolving into chromosomes (fig. 22). Throughout these stages there is no sign of a double filament; in fact the whole structure is indistinct. Figs. 23 and 24 show fully formed chromosomes which have split into daughter chromosomes. A side view of the spindle (figs. 23, 24) presents chromosomes apparently shaped like dumb-bells. A comparison of the above mentioned figures with fig. 25, a polar view of the equatorial plate, explains the actual condition. The daughter chromosomes are paired in the form of V's; the open ends of the V's are turned outward, the arms of the V's are nearly at right angles to the axis of the spindle. The appearance of the chromosomes of figs. 23 and 24, as described above, is due to the fact that only the tips of the chromosomes at the open ends of the V's can be seen; the seeming connection between the chromosome tips is occasioned by an indistinct view of the apices of the V's.

The separation of the daughter chromosomes as in the first division is not simultaneous. Fig. 23 shows a chromosome well on its way toward one pole before its sister chromosome, or the

[2] LAWSON in a recent paper (**23**) claims that the nuclear membrane does not break down or collapse as was formerly supposed to be the case. On the contrary, it behaves as a semi-permeable plasmatic membrane. Changes in the quantity and form of the chromatin previous to the metaphase are apparently accompanied by a change in the osmotic relations of the karyolymph. As a result of this there is a gradual decrease in the volume of the nuclear vacuole until the nuclear membrane closes in about the chromosomes; each chromosome becomes a single osmotic system.

other chromosomes, have moved far from the equatorial plate. Fig. 26 also shows chromosomes in the anaphase lagging behind at the equator, while the majority have nearly reached the poles.

CHROMOSOME NUMBER.—The metaphase of the second division represents the most favorable opportunity for the chromosome count. The chromosome number, however, was not determined with any finality. In diakinesis the chromosomes, though few in number, are so large that they obscure each other. A comparison of the counts attempted during diakinesis and the second metaphase places the haploid number of chromosomes at either 12 or 13.

Postsynapsis in the megasporangium

The difficulty of obtaining maturation stages of the megaspore mother cell discouraged a study of meiosis in the pistillate material. Of the many slides prepared, the majority showed synapsis; in addition only a few postsynaptic stages were procured. The nuclei of the megaspore mother cells are larger than those of the microspore mother cells, and when desirable stages are found they are exceedingly favorable for study.

The first stage noted after synapsis represents the nucleus as containing a mass of loosely interwoven filaments undivided and slightly beaded (fig. 28). The filaments thicken (fig. 29) and split longitudinally (fig. 30). Before the spireme breaks up into the bivalent chromosomes, it passes into strepsinema; the halves of the double filament draw apart, twist about, and cross each other (fig. 31). Attenuated portions of the spireme may be observed; transverse segmentation has begun and isolated pairs of chromosomes may be seen near the periphery of the nucleus.

In diakinesis the paired chromosomes occupy many positions with respect to each other, seldom lying strictly parallel. In fact, the description of diakinesis in the staminate loculus applies here. In fig. 33 several of the chromosomes offer a trace of a split, probably a precocious homotypic fission. In the microspore mother cells this split was not observed until the metaphase. This homotypic fission is described as occurring earlier in plants studied by the metasyndetists (MOTTIER, LEWIS, FARMER, DAVIS,

and others). MOTTIER (29) finds gemini in *Tradescantia* having double paired segments. This doubling is supposedly due to the reappearance of a split in the spireme which is believed to be a genuine homotypic fission and hence comparable to the split found in the paired segments of gemini in *Smilax*.

Mode of reduction

The mode of reduction in *Smilax herbacea* is essentially parasyndetic, though the procedure in the prophase seems to depart from the method described by GRÉGOIRE (17) for parasyndesis. According to GRÉGOIRE, the chromatin in the prophase takes the form of thin paired filaments (leptonema) which fuse (zygonema), shorten and thicken (pachynema), and again separate (strepsinema). In *Smilax* the chromatin in the prophase is distributed in granules, which are frequently seen in pairs. That which takes place between this condition and the spireme is obscured by synapsis. The construction and relative arrangement of the chromosomes in the spireme can be inferred only from subsequent behavior. After synapsis, two longitudinal splits occur; the first appears early in the spireme and can be traced through strepsinema to diakinesis; the second split shows sometimes in the univalent halves of the gemini at diakinesis, but more often not until the metaphase. From this we may infer that the first doubling is a separation of previously paired elements and that the chromosomes or chromatic bodies are placed side by side in the spireme. The second doubling is a genuine fission.

Discussion

PERSISTENCE OF CHROMOSOMES.—Much of the cytological work of recent years has brought forward directly or indirectly the question of the persistence of chromosomes or of some smaller unit. The rediscovery of the work of MENDEL has given added impetus to the hope of finding a physical basis for heredity or the unit characters of MENDEL which, according to experiment, seem to pass from generation to generation inviolate. Opinions concerning the existence of this cytological unit necessarily vary. Some favor the idea of persistent chromosomes; some maintain that

the unit is smaller; while the observations of others imply the non-existence of a persistent chromatin body.

The work of OVERTON strongly supports the theory of chromosome persistence. In the resting somatic nuclei of *Thalictrum purpurascens* and *Calycanthus floridus* the chromosomes are represented by definite visible bodies, the prochromosomes (OVERTON **33, 34**). From his study of the nuclei in the pollen mother cells of *Campanula grandis*, *Helleborus foetidus*, *Thalictrum purpurascens*, and *Calycanthus floridus*, he draws the conclusion that the chromosomes never lose their identity in either somatic or germ nuclei. Even on the spireme the chromosome unit is distinctly visible. During interkineses (OVERTON **35**) of somatic mitoses progressive vacuolation and enlargement of the chromosomes take place, but the chromosome outline can always be traced. LAIBACH (**21**) in working on the Cruciferae finds that the chromosomes remain as clearly defined in the resting condition as during mitosis.

ROSENBERG (**36**), in the resting nucleus of the hybrid *Drosera longifolia*×*rotundifolia*, finds paired chromatic bodies that equal the number of somatic chromosomes. These he calls prochromosomes or centers of chromosome formation. DAVIS (**9**) described chromatic bodies in the nuclei shortly after the last division in the archesporium of *Oenothera gigas*, which he thinks probably are chromatin centers or prochromosomes.

On the other hand, the theory of nucleolar origin of chromosomes does not support the view of chromosome permanence. The author has already referred to the work of WAGER (**43**) and DARLING (**7**) describing the budding of chromosomes from the nucleolus. SHEPPARD (**38**) investigated the behavior of the nucleolus in *Hyacinthus*. In the spireme stage he found the nucleolus apparently being drawn out upon the chromatin threads by means of nucleolar pseudopodia connected with the chromatin threads. Here, as described, the chromatin does not originate entirely from the nucleolus. BERGHS (**3**) found large nucleoli in the somatic cells of *Spirogyra* which break up into bodies partly chromatic and partly achromatic equal to the number of chromosomes. A more detailed citation of this will occur later. Miss DIGBY (**11**) states that there is no relation between the number of

chromatic aggregations in the resting nucleus of *Galtonia candicans* and the number of chromosomes; moreover, in the telophase of the somatic divisions the chromosomes lose their identity, their centers dissolving and the chromosomes breaking into small portions.

Many cytologists compromise on a middle ground and assume that bodies which are smaller than the chromosomes and into which the chromosome is divisible, are the chromatic units. These have been styled as pangens (MOTTIER **29**) or chromomeres (ALLEN **1**, LEWIS **24**). In the ordinary use of the above terms the pangen represents a smaller unit than the chromomere; in this connection the terms are used simply to designate a small chromatic body of no determined size. The chromosome represents a definite group of these units and is probably formed for the purpose of facilitating segregation and mitosis. ALLEN figures the actual union of chromatin granules in the spireme, with their subsequent separation. MOTTIER finds no evidence of prochromosomes but supports the theory of the individuality of pangens. LEWIS describes granules in the resting nucleus in excess of the number of chromosomes.

The differences among the above citations are not as serious as they might seem. By the adoption of a hypothetical unit smaller than the chromosome, it is not difficult to imagine that its appearance, whether alone or in close approximation to its fellows, might vary and vary much with the different species of plants studied. In the plants studied by OVERTON and LAIBACH the chromosomes may be said to pass from one stage to another always in definite uniform groups, the prochromosomes. We may say these bodies maintain their permanence because of an unchanging mutual attraction of the chromomeres in each chromosome. We may conceive of another condition in which the mutual attraction of the chromomeres in each chromosome group varies with the resting and active stages of the nucleus. Although ROSENBERG (**36**) found the somatic number of chromatin bodies in the resting nuclei of the hybrid *Drosera*, he considered them as chromatin centers about which chromatin units congregated in the prophase, always with the same relative arrangement. This theory advanced by ROSENBERG may be modified and extended to

cover a condition where no chromatin centers are visible, but in which the chromatin units, or small groups of units, arising from the fragmentation of a single chromosome, exert a mutual attraction and come together in a uniform body during the prophases.

It is somewhat more difficult to apply this conception to the cases of nucleolar origin of chromosomes. Although little is known about the nature and structure of the nucleolus, it seems plausible that the same relations between chromomeres may exist whether they are inclosed in a comparatively small body, the nucleolus, grouped in several small bodies, the chromosomes, or scattered in the nucleus.

An obstacle to the view of the persistence of either chromosomes or smaller chromatin units arises, however, when we consider the recent work of certain authors, such as that of Miss DIGBY (10) on *Galtonia candicans*, or that of GATES (16) on *Oenothera gigas*. Miss DIGBY describes a condition in *Galtonia* in which chromatin buds off from the nuclear framework, synaptic knot, or nucleolus, and passes into the cytoplasm or even into neighboring cells; these buds eventually disintegrate. Though Miss DIGBY implies that the parent nuclei develop normally, she does not describe their development beyond the spireme stage. However, she cites cases where entire loculi contained aborted pollen mother cells. GATES describes a similar phenomenon in *Oenothera gigas*. During the synaptic stage there is an extrusion of a part of the chromatic matter of the spireme into an adjoining cell; the extruded portion degenerates, but the nucleus from which it came behaves normally. CARRUTHERS (6), in a description of the cytology of *Helvella crispa*, states that there are extrusions of chromatin-like material from the poles of the nucleus, which disintegrate in the cytoplasm and take up nucleolar stains. GRIGGS (18) says, of the masses of chromatin in the nuclei of *Rhodochytrium*, a portion remains free and is cast into the cytoplasm or remains as beads on the spindle fibers, while the rest of the chromatin forms the chromosomes.

It is also not without interest to recall the condition of *Spirogyra* (BERGHS 3, MITZKEWITSCH 27) where a chromatic nucleolus during the anaphase shows tiny chromosomes enveloped in its mass; at the metaphase the nucleolus is divided and the halves move

toward the poles of the spindle where the nucleolar mass is resolved into large chromosomes. Upon decolorization, small portions, the size of the prophasic chromosomes, at the equatorial ends of the large chromosomes strongly retain the stain. The deeply stained portions of the large chromosomes BERGHS considers the "chromosomes veritables." In describing the larger bodies the word "chromosome" is used merely for convenience.

From the observations just noted, we may draw the conclusion that there are substances which are not chromatic in the sense of being the carriers of hereditary qualities, but which at some stages have the same appearance and pass through certain phases in closest proximity to the real chromosome. CARRUTHERS (**6**) suggests that the extruded bodies are masses of nutritive material for which the nucleus has no further use.

Conditions in *Smilax herbacea* do not support the theory of persistence of the chromosome as a physical unit, but of a smaller unit. The number of chromatin bodies in the microspore mother cell exceeds the number of somatic chromosomes; the same is true of the somatic nuclei of the nucellus. As these chromatin bodies vary in size, we consider that they are aggregates of units or chromomeres; also that the size of the chromomere aggregate varies with the number of units contained in it. Finally, during synapsis the chromomere aggregates form chromosomes according to a law of natural affinity. There is no evidence of a loss of chromatin or chromatin-like material.

PAIRING OF CHROMATIC ELEMENTS.—Closely connected with the theory of the persistence of chromatic units is the theory of the pairing of parental elements throughout the sporophytic phase. Before the theory of permanent, paired chromatic units was advanced, "pairing" was described only in connection with the prophase of the first meiotic division where parallel conjugation (parasynapsis or parasyndesis) of thin chromatic filaments was considered typical. A large group of cytologists now present a different mode of conjugation, namely, of the "end to end" type (telosynapsis or metasyndesis).

The difference between the two methods is not as important as it seems to be. Too much stress has been laid on the comparative

merits of parasyndesis and metasyndesis. Granted that the chromosomes are fully formed, so far as the arrangement of constituent parts is concerned, at the time of synapsis, there need be no difference in the ultimate result whether the homologous chromosomes appear in the spireme side by side or one ahead of the other; in either case the paired chromosomes are adjacent and are not prevented by their previous arrangement from exhibiting the same relations from diakinesis through succeeding stages. JORDAN (**20**) suggests that both parasynapsis and telosynapsis may occur in the same prophase; that is to say, the "end to end" arrangement of chromosomes in the spireme is frequently followed by a pronounced loop formation, resulting in a parallel approximation of chromosomes. On the other hand, parasynapsis may be followed by fusion of the ends of paired chromosomes (diakinesis). GATES (**15**) explains the appearance of both types of conjugation from a mechanical standpoint. According to his view short chromosomes are particularly adapted to telosynapsis, while long chromosomes are parasynaptic.

The extension of the theory of chromosome pairing to cover the entire sporophytic phase is supported by the observations of several investigators. STRASBURGER (**39**), in a study of the root tips of *Pisum*, found many cases where the chromosomes were grouped in pairs on the nuclear plate. He concludes that the parental chromosomes in the nuclei of the sporophyte generation do not form two separate groups, but that the homologous chromosomes occur in definite positions with respect to each other. He also figures a similar condition in an integument cell of the ovule of *Lilium Martagon* (STRASBURGER **40**). Miss SYKES (**42**) describes a paired arrangement of chromatic elements in the somatic nuclei of *Hydrocharis Morsus-ranae* and *Bryonia dioica*. *Lychnis dioica* and *Sagittaria montevidensis* show fully formed chromosomes lying in pairs. OVERTON (**34**) states that, in the somatic nuclei of plants which he has studied, definite chromatic bodies were visible lying in pairs (*Campanula grandis*, *Helleborus foetidus*, *Thalictrum purpurascens*, *Calycanthus floridus*). BONNET (**4**), however, finds no satisfactory evidence of pairing in the diploid nuclei of the *Yucca*, although he finds definite chromosomes.

In plants which do not possess the persistent chromosome, the chance of observing the paired arrangement of smaller groups of chromatic units is much lessened. As mentioned early in this paper, the chromomeres or chromomere aggregates in either somatic or germ nuclei of *Smilax herbacea* frequently appear in pairs or double pairs. This seems to occur too often to be merely a coincidence, yet it could not be determined as a universal condition.

THE SEX DETERMINANT IN PLANTS.—Although no reference to the sex determinant has been made in the preceding pages, this study was first attempted with the hope of finding the idiochromosome in a dioecious plant. WILSON (**44**), in his studies of the determination of sex in insects, places the decisive sex factor in the sperm. Here he found one-half of the sperms each carrying one or more extra chromosomes or a chromosome unique in size. All cases which cannot be placed in the above groups he relegates to a group where there is no physical variation of the chromosomes in the sperm cells, but where one may presume a physiological variation.

Botanists have been unsuccessful in their efforts to find the idiochromosome. DARLING (**7**) in working on the sexual cells of *Acer Negundo* (staminate material) found that one daughter nucleus after the first division contained a secondary chromatin mass; after the second division, two of the granddaughter nuclei each contained one more chromatin mass than the other two. In the resting stage, however, all the nuclei looked alike. To these secondary masses DARLING attached a possible sexual significance. Miss SYKES (**42**) found the nuclei of both sexes of unisexual plants she had studied (*Hydrocharis Morsus-ranae, Bryonia dioica, Lychnis dioica, Mercurialis perennis, Sagittaria montevidensis, Cucurbita Pepo*) to be identical in the number and form of the chromosomes. STRASBURGER'S (**41**) efforts to find a structural basis for the determination of sex were rewarded with negative results. On the nuclear reduction plate of *Melandryum rubrum* he observed a pair of chromosomes much larger than the other gemini; this same condition appeared again on the homotypic spindle. Thus, one large chromosome was distributed to each of the tetraspores.

However, he found that, though the four pollen grains of each group usually agreed in size, there were groups in which two of the pollen grains were larger than the corresponding two. This possible indication of sex differentiation was destroyed when he found a like condition among the pollen grains of hermaphrodite plants (*Lychnis Flos-jovis*, *Silene fimbriata*).

In *Smilax herbacea* no trace of an idiochromosome was found. During the metaphase of the first division one frequently found a chromosome pair which anticipated the other gemini in separating and moving toward the poles (figs. 17, 18), but no differences were observed in the homotypic division or in the tetraspores. Moreover, precocious chromosome movement away from the equator during the heterotypic division has been reported by CARDIFF (**5**) in *Salomonia*, a hermaphrodite.

For the present, then, we may assume a physiological difference in the tetraspores of dioecious plants which has no physical manifestation.

Summary

1. The nuclei of the young microspore mother cells each contain several nucleoli of varying size. The nucleoli fuse during the prophase, forming one large nucleolus at synapsis. During the early prophase the nucleolus is provided with several "papillae." These doubtless represent small nucleolar bodies which also fuse with the larger nucleoli. The nucleolus usually has at least one papilla until its disappearance at the metaphase.

2. The chromatin in the young microspore mother cell occurs in the form of granules or chromomere aggregates (the chromomere is here considered a chromatic unit).

3. There is no presynaptic reticulum, leptonema, or zygonema. The chromatin granules are held in an indefinite linin mesh.

4. Synapsis is reached by a contraction of the linin-supporting structure drawing the chromatin granules together.

5. The chromatic elements emerge from synapsis in the form of a spireme which soon becomes double.

6. The spireme shortens and thickens. Transverse segmentation of the spireme results in the formation of long paired chromo-

somes which continue to shorten and thicken, producing the characteristic gemini of diakinesis.

7. The separation of homologous chromosomes at the metaphase proceeds as usual. At this stage the chromosomes frequently show a split preparatory for the second division.

8. At the telophase a nuclear membrane appears. During interkinesis the chromatin is in the form of a band, apparently wound about the periphery of the nucleus. The band seems to be split or slightly vacuolate.

9. With the formation of the spindle of the second division the nuclear membrane disappears and the chromatic band resolves into chromosomes.

10. At the homotypic metaphase the longitudinal halves of the chromosomes separate.

11. The method of reduction in *Smilax herbacea* essentially coincides with the "hétérohoméotypique" scheme of GRÉGOIRE.

12. The persistent chromatic body in *Smilax* is a smaller unit than the chromosome.

13. The pairing of chromatic bodies was observed in the prophase, but not as a universal phenomenon. The same condition was evident in the nuclei of the nucellus.

14. An effort to find a sex determinant in *Smilax* was futile.

The writer wishes to express her indebtedness to Professor A. W. EVANS for suggesting this study and for his helpful advice and criticism.

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EXPLANATION OF PLATES IV–VI

Microspore mother cells

FIGS. 1 and 2.—Young spore mother cell; nucleoli fusing; ×2400.

FIG. 3.—Same as fig. 1; nucleolar "papillae"; ×2400.

FIG. 4.—Young spore mother cell; paired chromatin bodies; ×2400.

FIG. 5.—Spore mother cell with one papillate nucleolus; chromatin bodies in pairs; ×2400.

FIG. 6.—Early synapsis; ×1725.

FIG. 7.—Synapsis; ×1725.

FIGS. 8, 9.—Late synapsis; ×1725.

FIG. 10.—Spireme; ×1725.

FIG. 11.—Early spireme stage; ×2400.

FIG. 12.—Spireme; ×2400.

FIG. 13.—Late spireme; beginning of transverse segmentation of spireme; ×2400.

FIG. 14.—Strepsinema; ×2400.

FIG. 14*a*.—Pair of homologous chromosomes; same stage as fig. 14; ×2400.

FIG. 15.—Diakinesis; ×2400.

FIGS. 16–18.—Metaphase; ×2400.

FIG. 19.—Late anaphase; ×2400.

FIG. 20.—Interkinesis; ×2400.

FIGS. 21–26.—Homotypic phases.

FIG. 21.—Prophase; ×1725.

FIG. 22.—Late prophase; ×1725.

FIG. 23.—Metaphase; ×1725.

FIG. 24.—Metaphase; ×1725.

FIG. 25.—Metaphase; polar view of equatorial plate; ×1725.

FIG. 26.—Late anaphase; ×2400.

FIG. 27.—Nucleus from somatic cell of young ovule; ×2400.

Megaspore mother cells

FIG. 28.—Early spireme stage; ×2400.

FIG. 29.—Spireme; ×2400.

FIG. 30.—Spireme; later stage than fig. 29; ×2400

FIG. 31.—Strepsinema; ×2400.

FIGS. 32, 33.—Diakinesis; ×2400.

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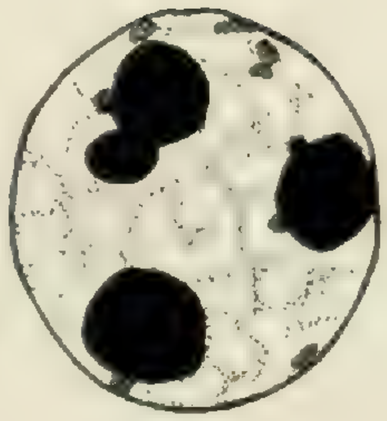

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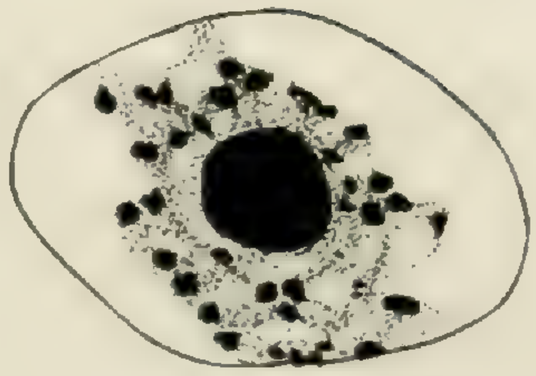

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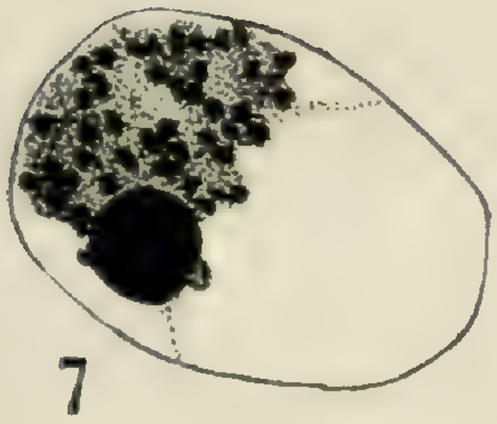

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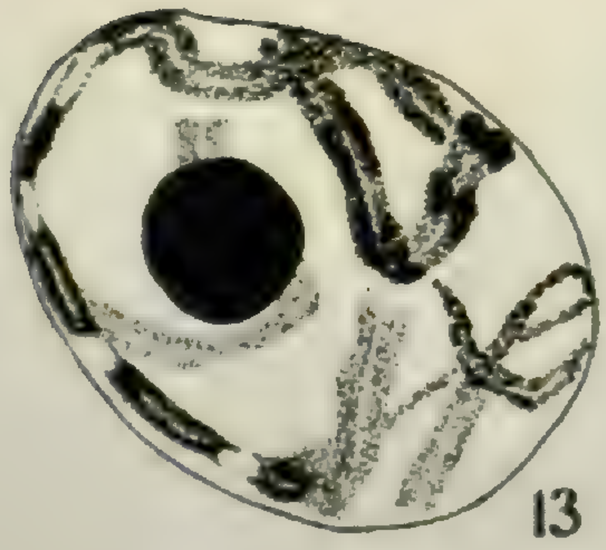

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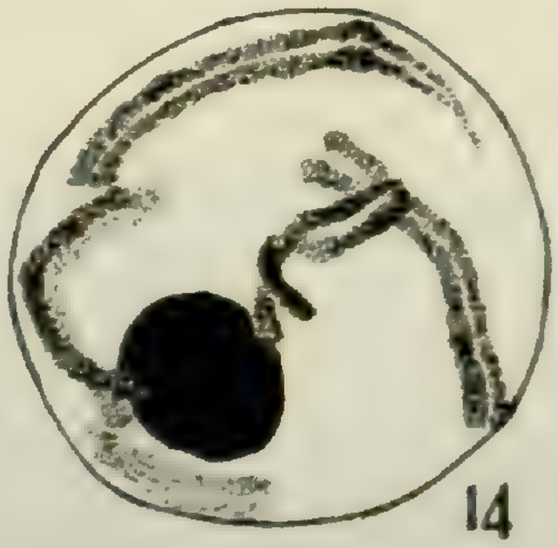

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ELKINS on SMILAX

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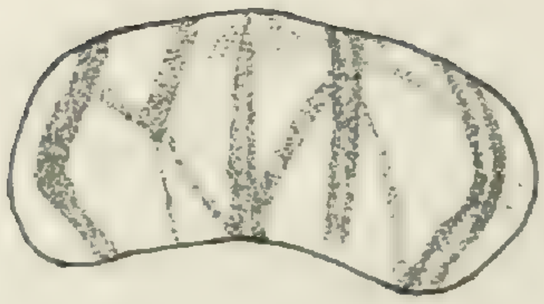

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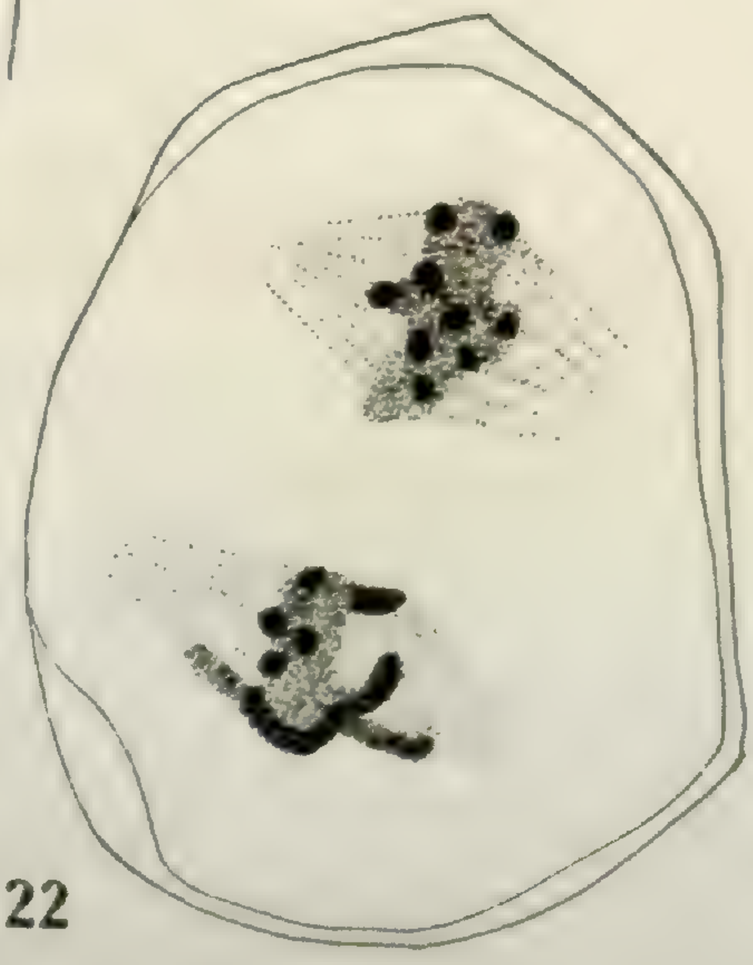

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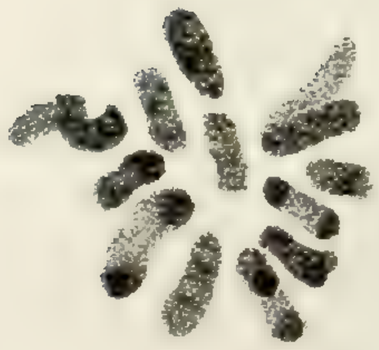

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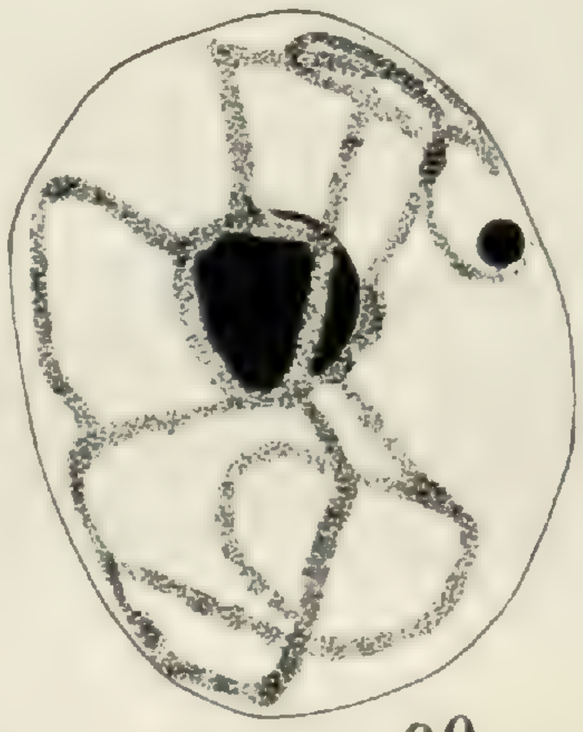

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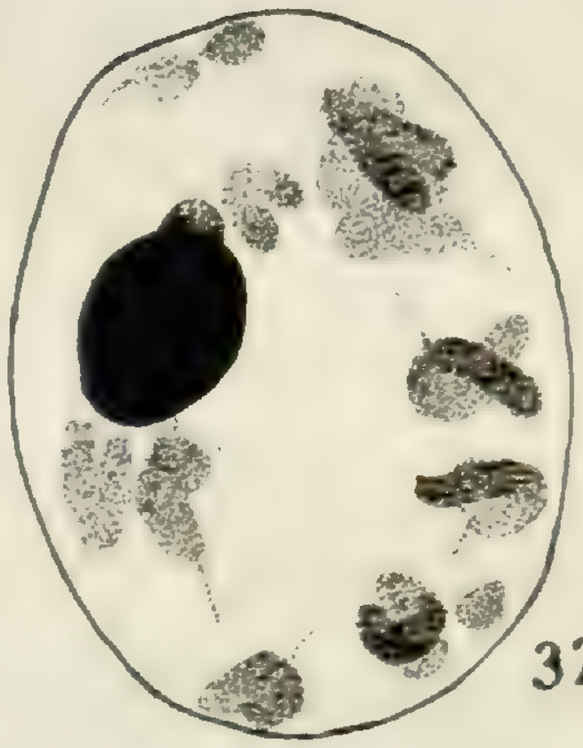

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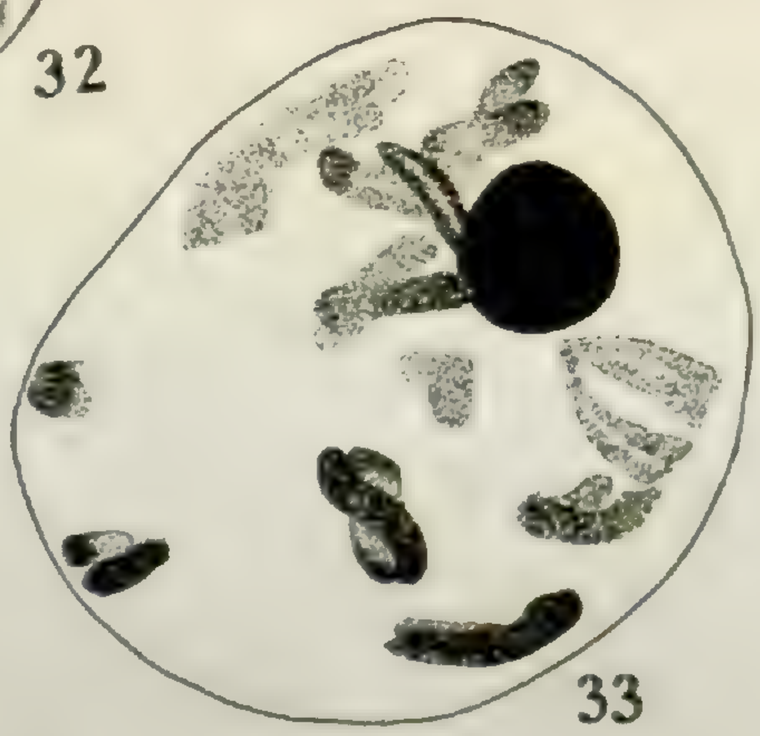

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ELKINS on SMILAX

COMPARATIVE HISTOLOGY OF ALFALFA AND CLOVERS

KATE BARBER WINTON

(WITH EIGHT FIGURES)

Perennial leguminous forage plants are growing in importance both for green feeding and for hay, and some of them, notably alfalfa, red and alsike clovers, are well adapted for grinding into meal. The work detailed in this paper was undertaken to facilitate the microscopic identification of the species named in mixed cattle foods.

The highest feeding value of the hay or "meal" is obtained from plants cut in early flower, though the more or less mature fruits and seeds are not infrequently found in the products on the market, especially in alfalfa meal.

Alfalfa

Alfalfa (*Medica sativa* [L.] Mill., *Medicago sativa* L.) is a native of Asia and has been cultivated for fodder since long before the Christian era. It is now grown in both hemispheres, especially in the arid and semiarid regions of the Southwest, for use either fresh or dried. As the hay is brittle, resulting, when fed from the bale, in a considerable loss of leaves, the product is often kiln-dried and ground to a meal.

FIG. 1.—Alfalfa (*Medica setiva*): *I*, leaf, ×1; *II*, flower, ×3; *III*, seed, ×3; *IV*, fruit, ×3.

Ordinary alfalfa, or lucerne, branches profusely and bears alternate leaves (fig. 1, *I*) consisting of three distinct obovate to lanceolate leaflets finely dentate at the apex. The plant is described as glabrous; hairs, however, are evident under a lens and are highly characteristic with higher magnifications. The flowers (fig. 1, *II*) appear in racemes of 8–25 each and wither after flowering. They are of the distinctly papilionaceous type, small (8–10 mm. in length),

and delicate in structure. The hairy calyx consists of a tube and 5 teeth-like lobes of about the same length as the tube, at the base of which is inserted the violet-colored corolla. The 10 stamens are combined in two sets; the ovary is one-celled with several ovules. At maturity the brown pods (fig. 1, *IV*) are coiled 2–4 times in close spirals, the diameter of the coil being about 4 mm. The greenish-brown seeds (fig. 1, *III*), up to 3 mm. in length, are somewhat kidney-shaped.

Many varieties of alfalfa, less widely grown, vary in flower color, through blue, white, and green, to yellow, and in number of pod coils, seeds, and leaflets.

HISTOLOGY

STEM (fig. 2).—The *epidermal cells* (*ep*) are several times longer than broad and arranged end to end in longitudinal rows interrupted frequently by stomata and their accompanying cells. The outer and inner walls are slightly thickened, the former having a cuticle with delicate striations evident in cleared preparations.

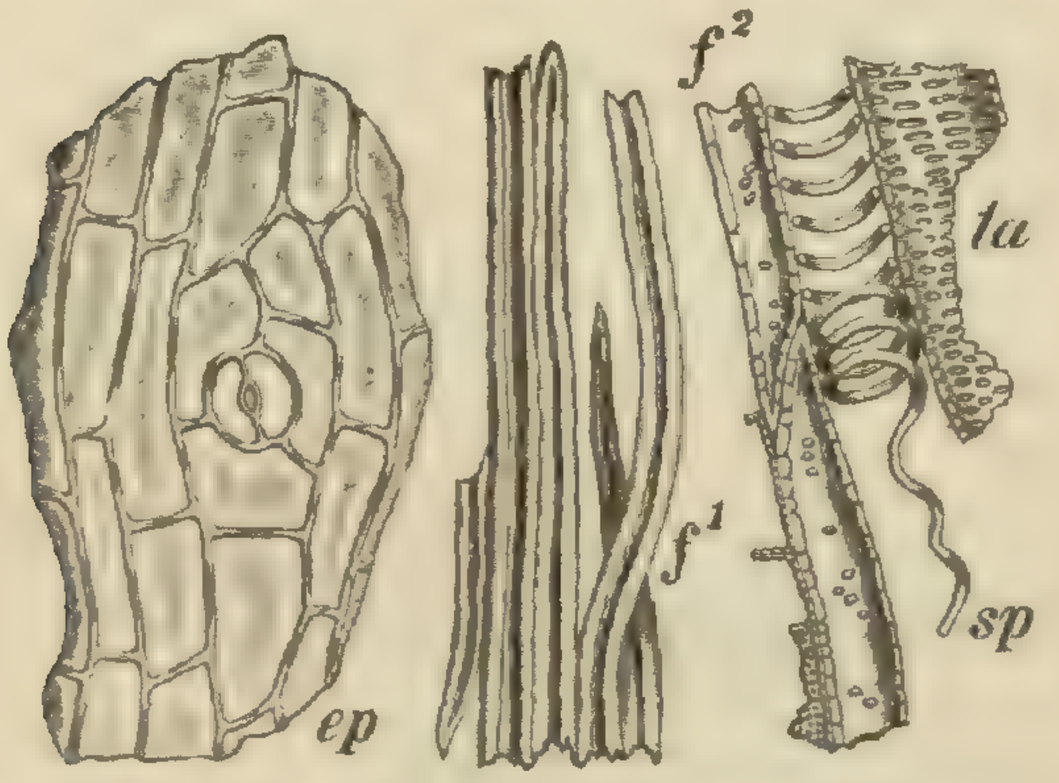


FIG. 2.—Alfalfa: elements of stem in surface view; *ep*, epidermis; f^1, bast fibers; f^2, wood fibers; *sp*, spiral vessels; *ta*, pitted vessels; ×160.

Bast.—Several layers of simple thin-walled chlorophyll-bearing parenchyma cells, interrupted occasionally, especially at the angles of the stem, by masses of collenchyma, form the outer tissues. Underlying this is a single layer of thin-walled crystal-bearing cells inclosing a zone of bast fiber bundles, each bundle being wedge-shaped in cross section. The individual fibers (f^1) are greatly elongated and have walls so strongly thickened that the lumen is often but a mere line.

Phloem.—This consists of a characterless mass of thin-walled cambium cells and sieve tubes.

Xylem.—The most evident elements of this woody tissue are the pitted (*ta*) and spiral (*sp*) vessels and the pitted wood fibers (f^2).

Pith.—This consists of comparatively large, thin-walled, pitted cells with no cell contents.

LEAF.—*Upper epidermis.*—The cells are approximately 35μ in diameter, although often elongated, especially over the veins. The cell walls are strikingly sinuous and of uniform thickness. Numerous simple stomata are scattered over the whole surface, and occasional hairs, similar to those so abundant on the lower surface, occur at the base of the leaf. Cuticular striations are very distinct in cleared material.

The *palisade layer* consists of very simple cells with breadth half the height.

Mesophyll.—The ground tissue of this layer is made up of a loose mass of parenchyma with no characteristic features; accompanying the simple bundles, however, are crystal-bearing cells (fig. 3, *cr*) of diagnostic importance. The latter are thin-walled and arranged more or less end to end in longitudinal rows. Each cell contains a single monoclinic crystal about 18μ in length, the facets of which often appear corroded.

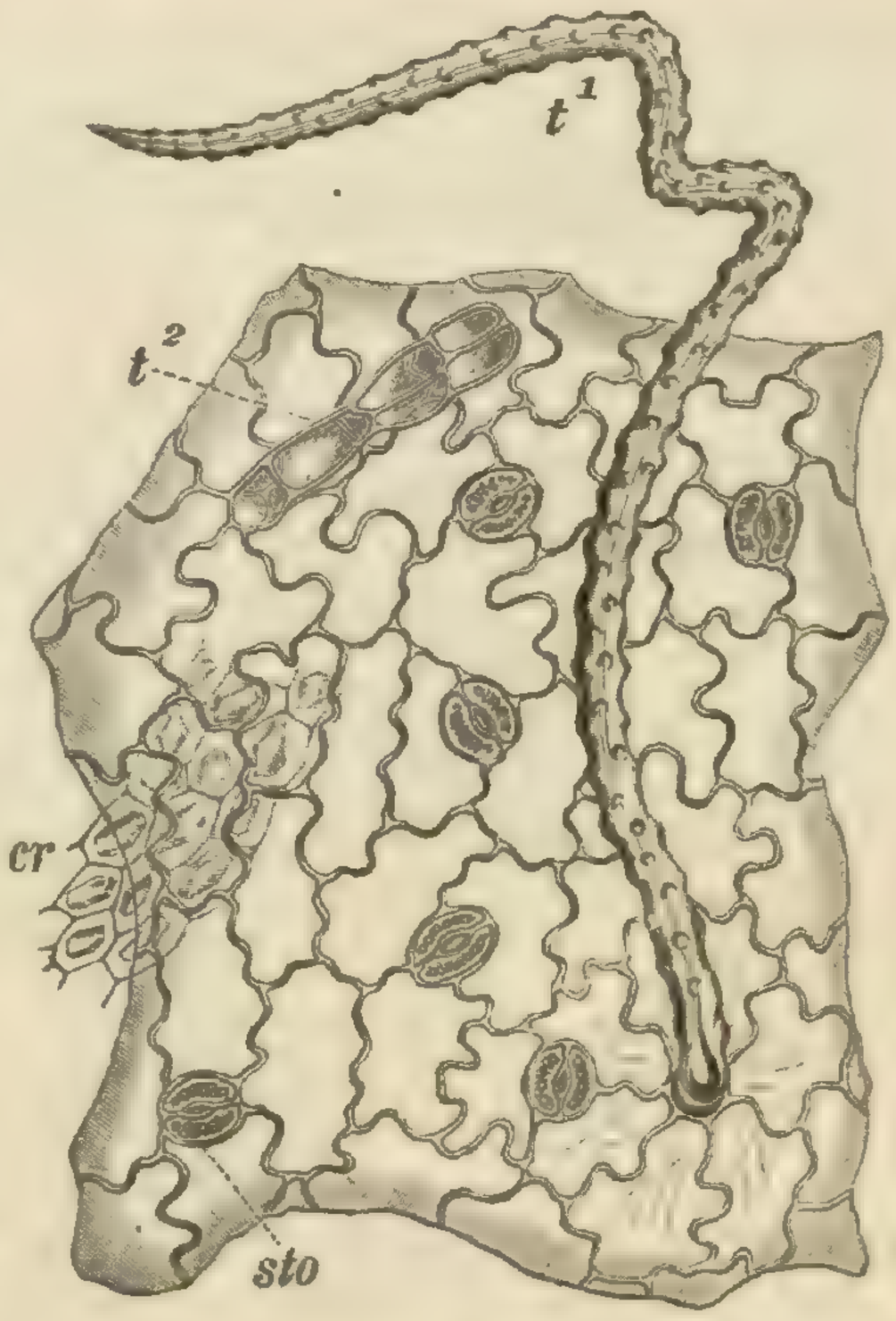


FIG. 3.—Alfalfa: lower epidermis of leaf with unicellular hair (t^1), capitate hair (t^2), and stoma (*sto*); *cr*, crystal cells accompanying bundles; ×160.

The *lower epidermis* (fig. 3) differs from the upper principally in the greater number of hairs which are scattered over the whole surface and margin of the leaf, being especially numerous along the veins. The cells surrounding the hair base form a rosette. The hairs are of two kinds, unicellular (numerous) and capitate (scattered). The unicellular hairs (t^1) are more or less sinuous, thick-walled, the lumen being a mere line, with small but prominent warts distributed over the entire length. They arise from a small, slightly thick-walled basal cell and average 800 μ in length and 15 μ in breadth, though the length varies up to over 1.5 mm. The

capitate hairs (t^2) consist of a stalk of two or three cells and a multicellular head, all the cells being thin-walled and frequently collapsed.

CALYX.—The *epidermis* bears unicellular and capitate hairs of the same general structure as those on the leaf. On the calyx tube the unicellular hairs are comparatively short and thick-walled, while on the lobes they are longer and thinner-walled, with correspondingly broader lumen. The simple bundle running out to the tip of each lobe is surrounded by a layer of crystal cells each containing a crystal averaging 18 μ in length.

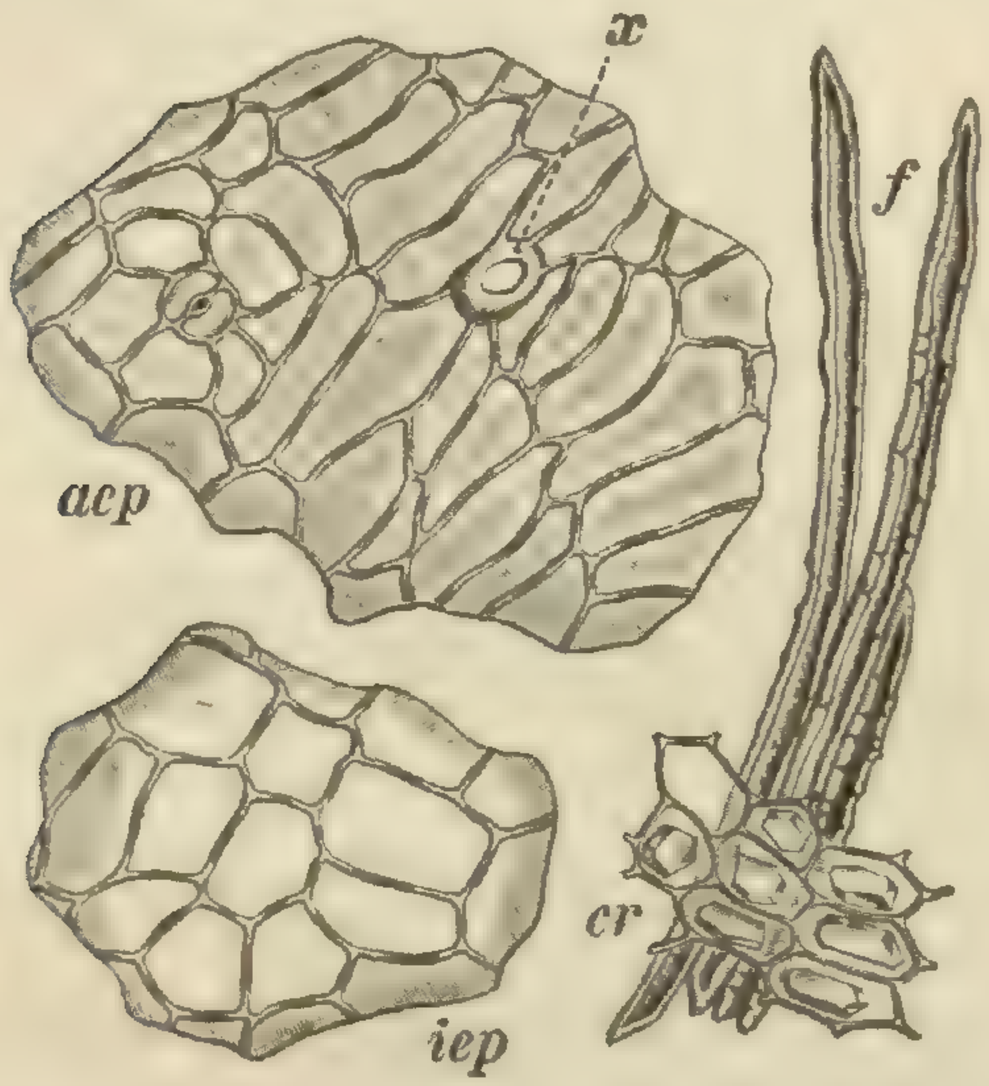


FIG. 4.—Alfalfa: elements of pod in surface view; *aep*, outer epidermis with hair scar (x); *iep*, inner epidermis; *cr*, crystal layer; *f*, fibers; ×160.

COROLLA.—The *epidermal cells* of the petals, at the base, are very thin-walled, elongated, and somewhat sinuous, and bear toward the tip papillae with striated cuticle. The bundles are very small, often but a single spiral vessel marking their course.

STAMENS.—The *filaments* consist of delicate cells similar to those of the petal in structure. The *anthers* have riblike thickenings over their whole surface.

PISTIL.—The *stigma* bears colorless papillae closely matted together.

The *style* is made up of small characterless cells except the outer half, which is covered with cells slightly thickened, apparently for mechanical support.

Ovary.—The small thin-walled epidermal cells bear numerous thin-walled unicellular and capitate hairs.

PERICARP (fig. 4).—The *epicarp* (*aep*) consists of a single layer of empty cells usually more or less elongated except at the stomata, about which they form a rosette. Hairs are frequently present, but often break off from the dried pod, leaving a scar (x) with a thickened wall.

Mesocarp.—The characteristic tissues are: crystal cells (*cr*)

with very thin walls, frequently side by side in rows, each cell containing a single crystal, and fibers (*f*) with rather blunt ends and pitted walls, the number of pits being most numerous in fibers with the thickest walls.

Endocarp (*iep*).—A single layer of epidermal cells without stomata completes the pericarp.

SPERMODERM (fig. 5, *S;* fig. 6).—The *palisade cells* (*pal*) are upward of 35 μ high and 8–10 μ broad, with rounded outer ends and a thin cuticle. A narrow light line (*l*), situated about 7 μ within the outer end, can be easily seen in cross section. As is

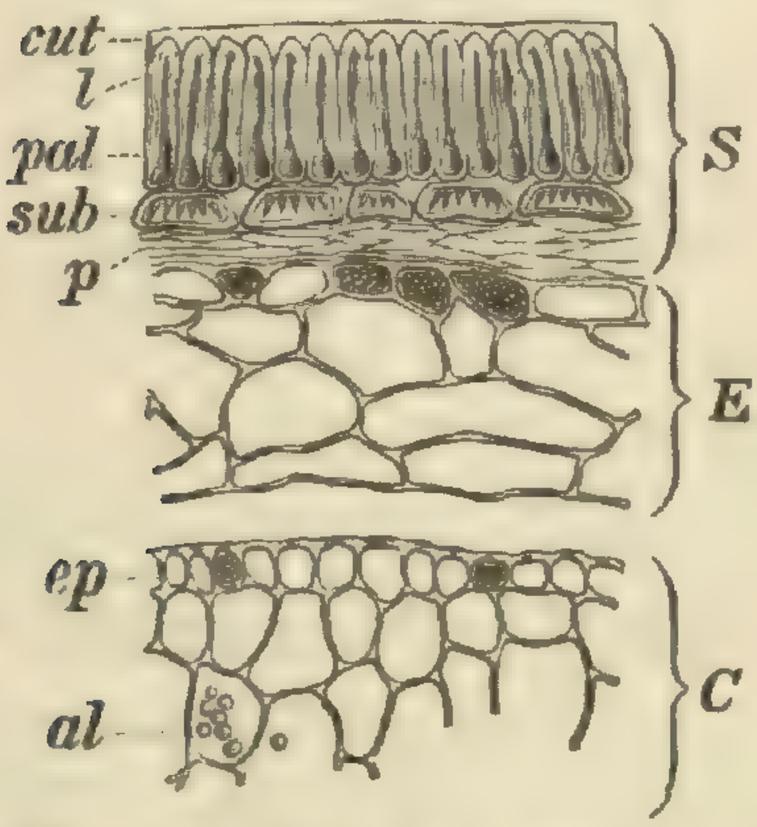


FIG. 5.—Alfalfa: seed in cross section; *S*, spermoderm consists of palisade cells (*pal*) with cuticle (*cut*) and light line (*l*), subepidermal layer (hour-glass cells) (*sub*), and parenchyma (*p*); *E*, endosperm; *C*, cotyledon with epidermis (*ep*) and aleurone grains (*al*); ×160.

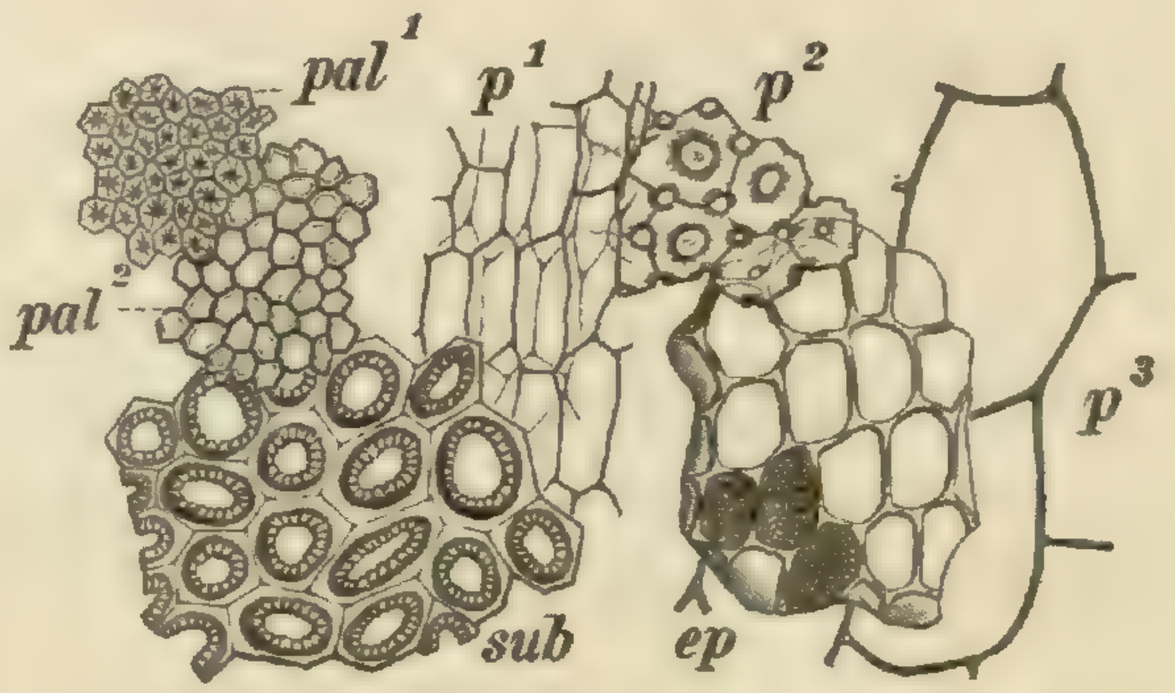


FIG. 6.—Alfalfa: elements of seed in surface view; *pal*¹, outer palisade cells; *pal*², inner palisade cells; *sub*, subepidermal layer (hour-glass cells), and *p*¹, *p*², parenchyma of spermoderm; *ep*, epidermis, and *p*³, parenchyma of endosperm; ×160.

usual in legumes, the outer cell walls are greatly thickened, showing a radiating cavity (*pal*¹) in surface view, while the inner portion of the cells has thinner walls and correspondingly broader lumen (*pal*²).

Subepidermal cells (*sub*).—These cells, although only about 6 μ high over the greater part of the seed, are very broad (upward of 30 μ) and are especially striking because of their prominent ribs clearly evident in surface view, where they present a beautiful radiating effect. In cross section they show evidence of the hour-glass form so characteristic of many legumes, the inner ends being broader than the outer.

The *parenchyma* (*p*) consists of several layers of compressed cells. The outer layers are of simple thin-walled parenchyma without intercellular spaces (p^1), while the inner layers are often distinctly spongy with evident intercellular spaces (p^2).

ENDOSPERM (fig. 5, *E;* fig. 6).—A simple epidermal layer (*ep*) containing aleurone grains is followed by several layers of large, more or less collapsed, empty cells with thin walls (p^3).

EMBRYO (fig. 5, *C*).—The cotyledons have a small-celled epidermis and mesophyll containing aleurone grains (*al*) but no starch. Palisade cells underlie the inner epidermis.

Red clover

Red clover (*Trifolium pratense* L.) is indigenous to Europe and is extensively grown for fodder in the United States, where it also grows spontaneously, having escaped from cultivation.

The pubescent stems are ascending, with 3-foliate toothed leaves, each oval leaflet often being notched at the apex and marked on the upper surface with a whitish spot. The rose-red flowers are borne in a dense sessile head closely surrounded by the uppermost leaves. The persistent calyx, with 5 bristle-like teeth and a bearded ring in the throat, is nearly as long as the delicate papilionaceous corolla, which is tubular below and withers after flowering. The pod differs from alfalfa in that it is one-seeded, straight, and flattened oval, with a very thin membranous lower half and hard caplike top. The seeds are slightly smaller than those of alfalfa, averaging 2 mm. in length. They are flattened kidney-shaped or rounded triangular with unequal sides one of which is concave. The color varies through light yellow to purple, the individual seeds being uniform in color or variegated.

HISTOLOGY

STEM.—The *epidermal cells* are longitudinally elongated with straight walls, often beaded especially just below the nodes, and a striated cuticle. Interspersed among these cells are numerous stomata and both unicellular and capitate hairs. The unicellular hairs, like those on the leaf, are long, thick-walled, warty, and

borne on a characteristic swelling of the epidermis having the appearance of an emergence.

Bast.—The only noteworthy tissues are the crystal-bearing cells accompanying the bundles of bast fibers and the large air spaces, below the unicellular hairs, such as occur on the leaf.

Phloem, *xylem*, and *pith* are similar to those of alfalfa.

LEAF.—The *upper epidermis* consists of approximately isodiametric cells with thin, gently wavy walls and scattered stomata. Hairs are absent.

Mesophyll.—The small bundles running through the simple parenchymatous ground tissue are accompanied by crystal-bearing cells, each cell containing a monoclinic crystal averaging 16 μ in length.

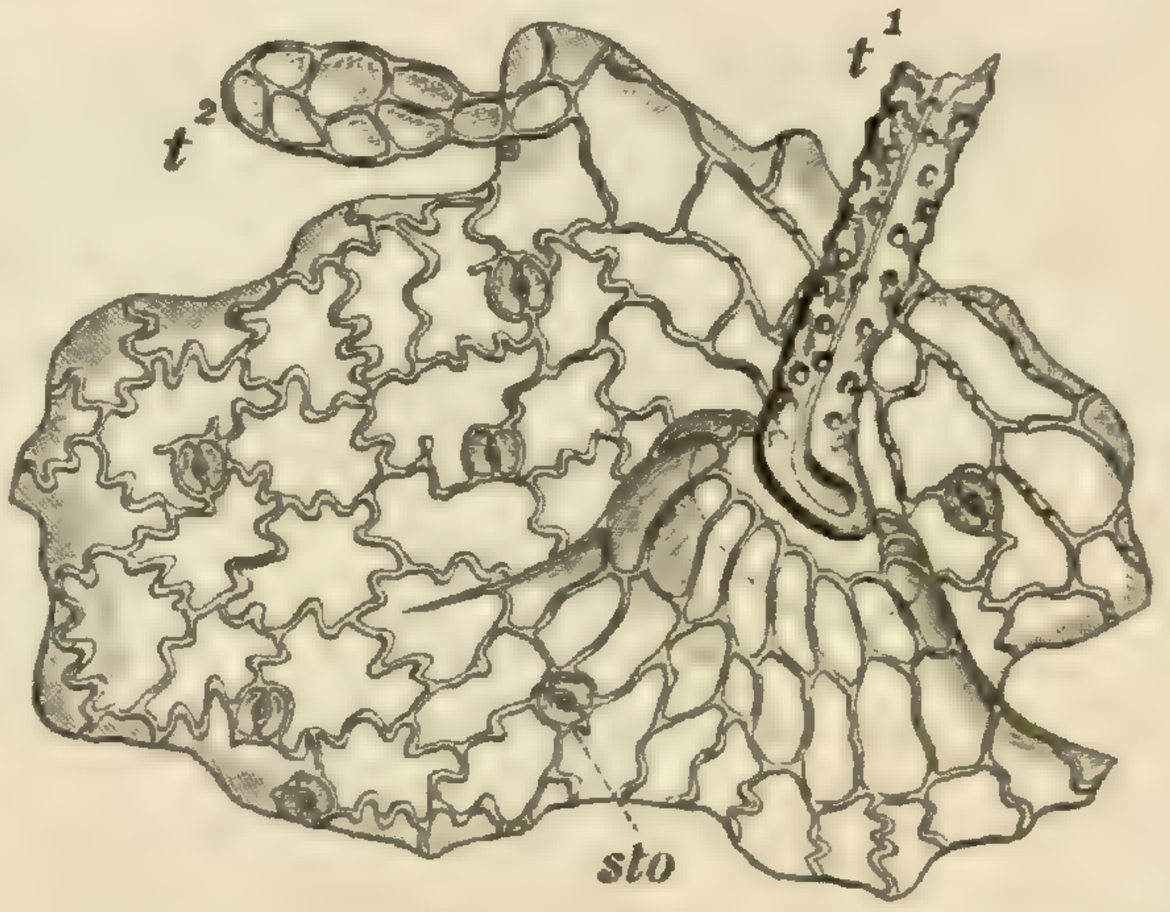


FIG. 7.—Clover (*Trifolium pratense*): lower epidermis of leaf with unicellular hair (t^1) arising from swelling of epidermis; t^2, capitate hair; *sto*, stoma; ×160.

The *lower epidermal cells* (fig. 7) have sinuous walls, the rather sharp bend of the waves being thickened and sometimes extended into the cell cavity as projections. Highly characteristic are the hornlike projections about the stomata (*sto*). The walls become slightly thicker and usually pitted toward the base of the leaf, especially over the veins. As on the stem, there are two forms of hairs, unicellular and capitate. The unicellular hairs (t^1) are stiff, very thick-walled and warty, varying in length up to 2 mm. and in diameter up to 30 μ. The warts are rather more prominent than on the corresponding hairs of alfalfa. They arise from a conical rosette of elongated cells over a large intercellular space, resembling in outward appearance an emergence. The capitate hairs (t^2), like those on alfalfa, consist of a stalk formed of a few cells in a single row and a club-shaped multicellular head.

CALYX.—The *outer epidermis* consists of simple cells with occasional wavy walls and numerous hairs both unicellular and capitate,

similar to those on the leaves and stem. The bristles, with papillae the whole length, end with a tuft of stiff unicellular hairs.

Mesophyll.—A single layer of crystal-bearing cells is conspicuous.

The *inner epidermis* is made up of wavy-walled cells and occasional hairs.

COROLLA.—The *epidermal cells* have very thin walls with papillae and striated cuticle toward the tip.

PERICARP.—The *epicarp* consists of sinuous-walled cells with scattered stomata. On the stem end the cell walls are thin, changing abruptly toward the tip to greatly thickened, sclerenchymatized and pitted walls.

The *mesocarp cells* are inconspicuous, with the exception of occasional scattered crystal-bearing cells.

SPERMODERM.—The *palisade cells* average 45 μ in height (running up to 55 μ over the radicle) and 7 μ in breadth. A narrow light line lies about 7 μ below the thin cuticle. They differ from the corresponding cells of alfalfa in that they are higher and the outer ends are flattened.

The *subepidermal cells* vary in height, but average 10 μ. They are upward of 20 μ broad and constricted in the center with lower ends broader than outer.

The *parenchyma* consists of thin-walled collapsed cells.

The ENDOSPERM and EMBRYO are of simple structure of no diagnostic importance.

Alsike clover

Alsike clover (*Trifolium hybridum* L.), although indigenous to Europe, has become very common in America. The plant branches, with erect stems bearing 3-foliate toothed leaves on long petioles and pedicellate flowers forming a loose round head on a long peduncle. Like alfalfa, the plant is described as smooth, though hairs are evident under a lens and are of diagnostic importance with higher magnifications. The membranous 5-cleft calyx is much shorter than the delicate rose-pink tubular corolla, which after flowering becomes brown and withering-persistent. The pod differs from that of alfalfa in that it is straight and from red clover in that it is 2–4-seeded. The greenish brown seeds are smaller

than those of alfalfa and red clover, reaching a length of 1.5 mm., but in shape resemble closely those of red clover. They are flattened rounded triangular with one concave side.

Histology

Stem.—The *epidermal cells* are thin-walled, pitted, and longitudinally elongated, with numerous stomata. The cuticle shows longitudinal striations. Occasional hairs both unicellular and capitate are present, the warts usually being indistinct.

Bast.—Conspicuous crystal cells are found in the bast just below the chlorophyll-bearing cells.

Leaf.—The *upper epidermis* consists of isodiametric cells, averaging 30 μ in diameter, with straight thin walls.

In the *mesophyll* the cells accompanying the bundles contain crystals averaging 15 μ in length.

Lower epidermis (fig. 8).—The cells are similar to those of the upper surface, the walls toward the leaf margins becoming gently wavy. Occasional unicellular (t^1) and capitate (t^2) hairs are present, the former being indistinctly warty and arising from a slightly thickened epidermal cell. They vary in length up to 800 μ.

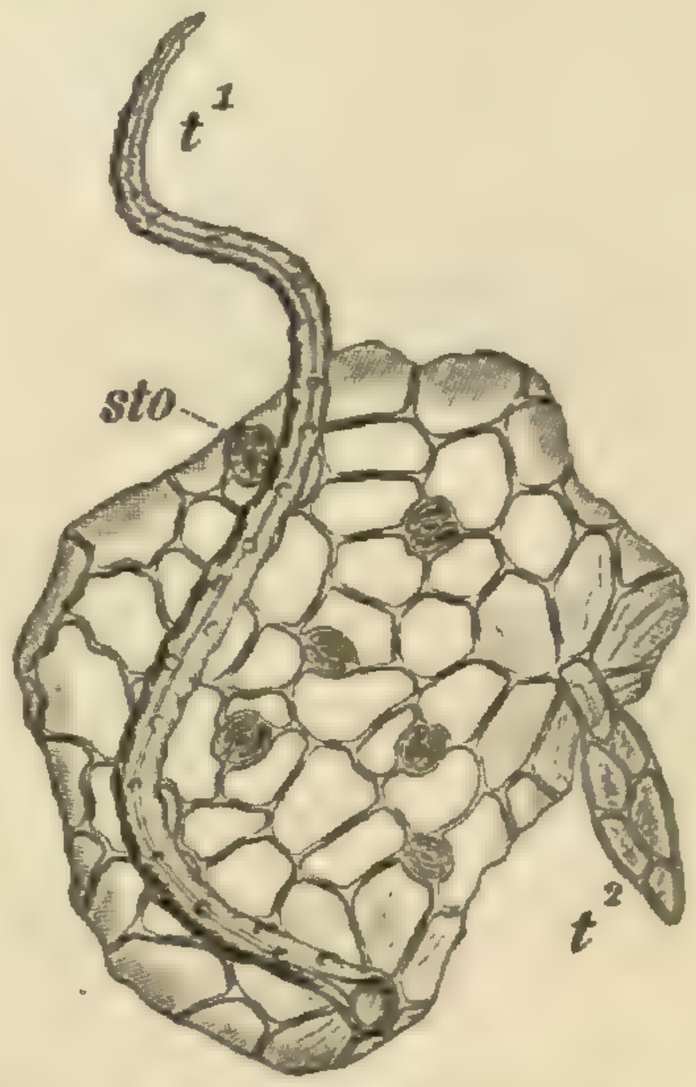


Fig. 8.—Alsike clover (*Trifolium hybridum*): lower epidermis of leaf with unicellular hair (t^1), capitate hair (t^2), and stoma (*sto*); ×160.

Calyx.—The *outer* and *inner epidermis*, with sinuous walls, bear capitate hairs similar to those on the leaf, also, at the base of the lobes and along their margins, unicellular thick-walled hairs with occasional indistinct warts.

Corolla.—The *epidermis* consists of elongated sinuous-walled cells with striated cuticle, papillae being present at the tip of the petals.

Pericarp.—The *epicarp* consists of transversely elongated slightly sinuous thin-walled cells, scattered stomata, and, especially at the margins, capitate hairs.

The *mesocarp* is but a few cells thick, except at the margins. Scattered crystal-bearing cells occur either singly or in groups.

The *endocarp* is made up of a single layer of thin-walled elongated cells.

SPERMODERM.—The *palisade cells* are 30–50 μ in height and 7 μ in diameter, with a narrow light line about 7 μ from the outer end. They differ from the palisade cells of alfalfa in that they are slightly higher, and from those of red clover in that they are rounded (not flattened) on the outer ends.

The *subepidermal cells* are not distinguishable from those of alfalfa and red clover.

The *parenchyma* consists of thin-walled collapsed cells.

The ENDOSPERM and EMBRYO are of simple structure of no diagnostic importance.

Identification in ground material

In a coarsely ground product, fragments of the leaves, flowers, pods, and seeds may be picked out and identified, but when powdered the unicellular hairs and crystals are the most conspicuous

	Alfalfa	Red clover	Alsike clover
Lower epidermis of leaf.....	Wavy walls	Deeply sinuous walls with projections at angles and about stomata	Straight walls
Unicellular hairs.....	Average diameter 15 μ, warts prominent	Average diameter 30 μ, warts prominent, arising from epidermal swelling	Average diameter 13 μ, warts indistinct

elements. Red clover may be distinguished from alfalfa and alsike clover by its larger, stiffer, and more numerous unicellular hairs arising from a swelling of the epidermis; alsike clover, from alfalfa and red clover, by the less distinct warts on the unicellular hairs.

The cell walls of the lower epidermis of the leaf are also characteristic, those of alsike clover being straight, of alfalfa simply

wavy, and of red clover very sinuous with projections at the angles and about the stomata.

A scheme for the identification of these three legumes by means of the epidermal cells of the leaf and the unicellular hairs is given in tabular form on the preceding page.

The palisade cells of the seed in alfalfa are not over 35 μ high, whereas in alsike and red clover they average somewhat higher. In red clover the outer ends of these cells are flattened, but in alfalfa and in alsike clover they are rounded.

CHICAGO, ILL.

THE RÔLE OF OXYGEN IN GERMINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 181

CHARLES A. SHULL

A study of the respiration of *Xanthium* seeds was undertaken some time ago with the purpose of determining whether there was any change in the permeability of the seed coats to oxygen during the period following the normal ripening of the seeds. Some evidence was noted previously[1] that there was either a change in permeability of the seed coat, or a change in oxygen need of the embryo during the early winter, and it was believed that a careful measurement of the oxygen used by the seeds with coats on and off at successive intervals during the year would show which of these changes occurred, and at what period of the ripening process. Circumstances have prevented the carrying out of this series of tests; but the preliminary results are of sufficient interest in connection with the rôle of oxygen in germination behavior to warrant placing the data on record. The measurements were made with a respirometer of excellent type designed by Dr. WILLIAM CROCKER, to whom I am further indebted for suggestions regarding the problem. The respirometer was kept in a Freas thermostat at 25.25° C., and the volumes of oxygen used are reduced to standard conditions. Seeds of *X. glabratum* in dry storage for nine months were used.

First it was necessary to know what part of the oxygen was used by the coats under ordinary atmospheric germinative conditions. Two lower seeds were placed in one chamber of the respirometer, and the coats of two lowers in the other chamber. In 22.5 hours the two seeds used 0.475 cc. of oxygen, while the two coats used 0.098 cc. From the results of BECQUEREL'S work[2] I had suggested that the coats were probably responsible for a part

[1] SHULL, CHAS. A., The oxygen minimum and the germination of *Xanthium* seeds. BOT. GAZ. **52**:453–477. 1911.

[2] BECQUEREL, PAUL, Recherches sur la vie latente des graines. Ann. Sci. Nat. Bot. IX. **5**:193–320. 1907.

of the respiration of intact seeds. That part is now shown to be considerable, amounting in this instance to 20 per cent of the total.

The respiration of lower and upper seeds with coats intact under atmospheric conditions of germination was compared, with the result that two lowers used 0.687 cc. and the two uppers 0.509 cc. of oxygen in 42.3 hours, a ratio of lowers to uppers of 1.35:1. It should be said that upper seeds always weigh less on the average than the lowers; and in using equal numbers of seeds the weight of respiring substance is somewhat less in the uppers.

The respiration of the lowers with coats on and coats off in ordinary atmosphere is especially interesting (see table I).

TABLE I

Time	Oxygen used		Ratio
	Coats off	Coats on	
10 hours.....	0.500 cc.	0.3615 cc.	1.38:1
10–15 "	0.478 "	0.1025 "	4.65:1
15–17.25 "	0.316 "	0.040 "	7.9 :1
Total.....	1.294 "	0.504 "	2.57 :1

TABLE II

Time	Oxygen used		Ratio
	Coats off	Coats on	
5 hours..... ...	0.1609 cc.	0.075 cc.	2.14:1
5–15 "	0.618 "	0.1107 "	5.58:1
Total, 17.25 hours...	0.9825 "	0.1947 "	5.046:1

A similar test with uppers is shown in table II. The coats were not placed in the chambers with the embryos where coats were off, so that the ratios in the tables are too low as regards actual embryo respiration. The rapid increase of use of oxygen by naked embryos as germination commences is well illustrated.

Finally, the oxygen used by uppers and lowers with coats on, in an atmosphere 96 per cent oxygen, was compared, with significant results. Two lower seeds used 1.007 cc. in 12.5 hours, while the

uppers used but 0.4406 cc., a ratio of 2.28:1. A repetition of this experiment resulted in the lowers using 1.257 cc. in 22 hours, the uppers 0.772 cc., a ratio of 1.63:1. Invisible defects in coats might cause some variation in these ratios, but they are believed to approximate average results.

It is interesting to note that two lowers in atmosphere used 2.687 cc. of oxygen in 42.3 hours, while the same number used 1.007 cc. in 12.5 hours in 96 per cent oxygen; and that uppers which used 0.509 cc. in 42.3 hours used 0.4406 cc. in 12.5 hours in 96 per cent oxygen. The increased oxygen pressure causes a large increase in the oxygen intake of both seeds with coats intact, but exerts the greater influence on the lowers. The relation between oxygen influence and respiration seems to be close. At least we may say that the conditions of the oxygen supply which lead to increased use of oxygen are just the conditions which bring about germination. The possibility that oxygen exerts its stimulative effect on germination by increasing respiration, thereby yielding more energy, is strongly suggested, without, however, precluding the possibility that other effects correlated with increased respiration might determine its influence in germination.

BECKER[3] recently tested the influence of oxygen on the germination of seeds of several plants. The fruits of *Dimorphotheca pluvialis* were found to germinate more readily in O_2 than in air, the ray seeds especially showing the favorable influence. Short exposures to oxygen (15 hours) had no such effect, but if the time of exposure were lengthened to 30 hours, this exposure favored further germination under atmospheric conditions. The ray seeds again showed the effect more strongly than the disk seeds. When the fruit and seed coats were removed, 10 hours' exposure to oxygen affected germination favorably, but 13 hours' lengthened the germination time. The seeds of *Calendula eriocarpa* with coats intact were greatly favored by oxygen, while the yellow-brown vertical fruits of *Atriplex hortensis* and *A. nitens* showed an injurious effect from increased oxygen pressures. The relation of oxygen to germination in these cases seems to be irregular and inconstant.

[3] BECKER, H., Über die Keimung verschiedenartiger Früchte und Samen bei derselben Species. Beih. Bot. Centralbl. **29**[1]:21–143. 1912.

BECKER draws a general conclusion from his results that oxygen acts as a stimulus, and takes particular exception to the idea advanced by CROCKER[4] that the oxygen increases the respiration and in this way initiates germination. In reviewing BECKER'S paper,[5] I stated that there was no doubt that in *Xanthium* the oxygen was actually used in germination, and suggested that increased respiration might be identical with BECKER'S stimulus. While it is entirely possible that the oxygen influence is exerted through some other process correlated with increased respiration, the data presented here give the ground upon which that suggestion was based. Unfortunately, BECKER'S work gives us no data as to the respiration behavior of the seeds on which he worked, so that no comparison with the behavior of *Xanthium* seeds can be made at present.

LEHMANN,[6] in discussing the possibility that O_2 might act as a catalyst, accepts BECKER'S idea that oxygen acts as a chemical stimulus, not merely by increasing respiration. Of course, the word "stimulus" is vague and indefinite. But it should not imply an additional absorption of oxygen, for this could not occur without involving oxidation of some kind, which would be respiration. Even if oxygen is conceived to be a catalyser, that conception does not involve increased use of O_2, for catalysts are not used in the processes they carry on.

The biological rôle of oxygen is so complex that we may not say its effect is always due to increasing respiration or oxidation. The rôle, indeed, may be different in different seeds and plants. For instance, ÁRPÁD PÁÁL[7] claims that reduction of oxygen pressure even to 0.75 normal lengthens geo-presentation and geo-reaction time to a marked degree. This work of PÁÁL'S still awaits confirmation. And although the earlier work of STICH, JOHANNSEN, and others indicates that this amount of reduction

[4] CROCKER, WM., The rôle of seed coats in delayed germination. BOT. GAZ. 42:265–291. 1906.

[5] BOT. GAZ. 54:433. 1912.

[6] LEHMANN, E., und OTTENWÄLDER, A., Über katalytische Wirkung des Lichtes bei der Keimung lichtempfindlicher Samen. Zeitschr. Bot. 5:337–364. 1913.

[7] PÁÁL, ÁRPÁD, Analyse des geotropischen Reizvorgangs mittels Luftverdünnung. Jahrb. Wiss. Bot. 50:1–20. 1911.

should not affect the rate and nature of respiration, yet it would be very desirable to repeat PÁAL'S work, studying the rate of respiration along with presentation time and reaction time to discover whether there is a parallel effect with that on the respiration rate and germination power of seeds.

ZALESKI[8] has noted the influence of oxygen on the rate of protein synthesis. IVANOFF[9] has shown that oxygen is necessary for the transformation of zymogen into zymase, and, as is well known, there are a number of oxygen carriers and oxygen absorbers in the living cell. Xanthophyll and other pigments absorb oxygen, lecithin plays a similar rôle, and PALLADIN[10] has now shown that his respiratory chromogens take up oxygen readily. All of these facts go to show how complex the oxygen rôle may be, and suggest some of the possibilities of even brief exposure of seeds to oxygen.

On the other hand, however, it would be strange if the oxygen effect in some cases were not due simply to its influence upon respiration. The influence of the amount of oxygen present on aerobic and anaerobic respiration, which differ so markedly in the amount of energy released, is well known. Anaerobic may change over to aerobic on access of oxygen, with a consequent rapid rise in energy releasal leading to germination.

With these *Xanthium* seeds it has been shown that when the oxygen supply is increased, it in some way brings about an immediate and rapid increase in the rate of oxygen absorption. At the same time, the increased oxygen supply brings about an immediate germination of the seeds. The two effects, increased absorption and germination, are closely correlated as regards their relation to the oxygen supply. This shows conclusively, I believe, that the assumption made by BECKER and LEHMANN, which led them to reject the idea that the influence was exerted through increased respiration, is not correct, so far as *Xanthium* is concerned. Nor does their work throw any light on this particular point, since they

[8] ZALESKI, W., Zur kenntnis der Stoffwechselprocesse in reifenden Samen. Beih. Bot. Centralbl. **27**:63–82. 1911.

[9] IVANOFF, L., Über die sogenannte Atmung der zerriebenen Samen. Ber. Deutsch. Bot. Gesells. **29**:563–570. 1911.

[10] PALLADIN, W., and TOLSTAJA, Z., Über die Sauerstoffabsorption durch die Atmungschromogene der Pflanzen. Biochem. Zeitschr. **51**:381–397. 1913.

did not measure the respiration of the seeds on which they worked. However, owing to the complexity of the oxygen rôle in physiological processes, it is very difficult to say just which function or functions are affected. It seems certain that the oxygen acts as a limiting factor on some function, whether by limiting the process of respiration or energy releasal, by limiting enzyme formation or the action of oxygen carriers, or in other still less definite ways. The exact method by which absence of oxygen delays germination can be determined only by further investigation. In the meantime theories may well await the facts which will make philosophical discussion of this question unnecessary.

UNIVERSITY OF KANSAS
LAWRENCE, KAN.

BRIEFER ARTICLES

A METHOD OF HANDLING MATERIAL TO BE IMBEDDED IN PARAFFINE

(WITH ONE FIGURE)

The usual method of handling material to be imbedded in paraffine is slow and tedious, and attended with some danger of damage to the material. Ordinarily, each batch is killed in a separate bottle, in which it remains up to and even through the paraffine bath. In washing, a cloth is usually tied over the end of the bottle, which is put under a running tap or thrown into a vessel of running water. Either way results in washing of questionable thoroughness. Then the various grades of alcohols and xylols are pipetted on and off, a very tedious process with small light objects that are likely to be drawn into the pipette and injured or lost.

After trying several devices, the writer has had success in eliminating these difficulties by the following method: About 6.25 cm. lengths are cut from ordinary medium thickness glass tubing of the desired diameter. One end is heated and slightly flared. Over the flared end is tightly tied a piece of thin cloth, and the excess of cloth and string closely trimmed away (fig. 1, *A*). With a stout cord, a slip knot (*B*) is quickly tied and drawn up, and holds the cloth firmly. The material is placed in this cloth-bottom tube, and remains in it through all the processes up to the paraffine bath. If the objects are large or numerous, it is better to kill in an ordinary bottle and transfer to the tube just before washing.

In washing, the tube of material is placed in a vessel of less height (such as a small salt mouth bottle), and water siphoned into the tube from a supply vessel directly under the running tap (*C*). The tube end of the siphon is drawn out fine, allowing only a small amount of water to pass over. A section of rubber tubing in the siphon gives greater flexibility. Because the water level is high in the tube, and all the fresh water flows over the material, washing is very thorough. Under all conditions the stream of water is uniform and gentle, even with considerable variation in tap pressure, as we have in Allahabad. Any number of tubes, each with a separate siphon, may be grouped around the supply vessel and washed at one time.

The series of alcohols and xylols is prepared in tightly stoppered bottles and arranged in sequence. The tubes of washed material are placed in the bottle of lowest grade alcohol and transferred at proper intervals from bottle to bottle through the series. Any number of tubes are easily and rapidly handled by means of a pair of forceps. There is no danger of losing small objects, because the top of the tube is always

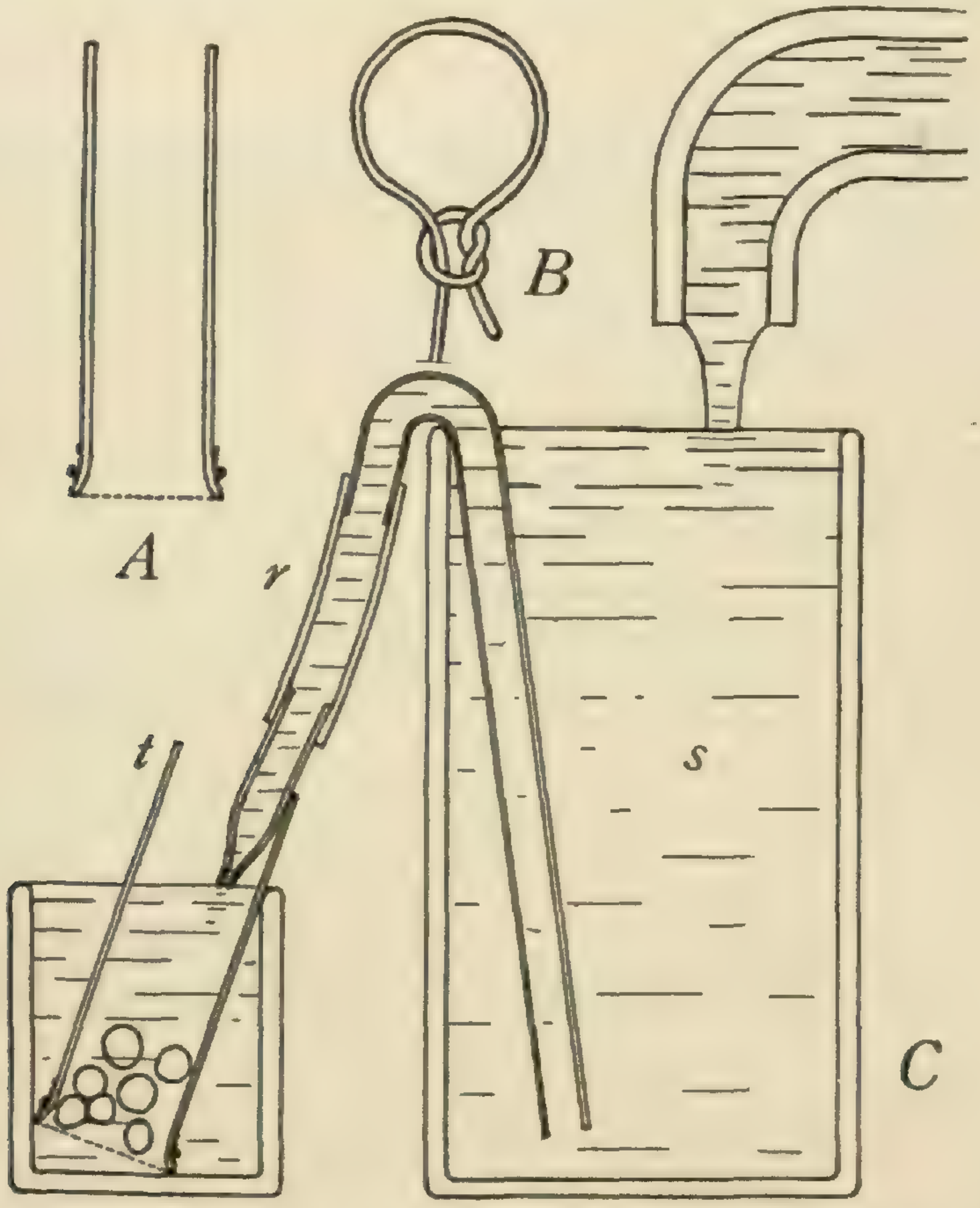


FIG. 1.—Sectional diagrams: *A*, end of tube flared and covered with cloth; *B*, rapid method of tying cord; *C*, arrangement for washing material; water flows from the tap into the supply vessel (*s*), through the siphon into the tube of material (*t*), through the cloth bottom, and overflows from the small vessel; a piece of rubber tubing (*r*) in the siphon adds flexibility.

above the level of the reagent. With proper labels on the series bottles, the exact location of the material, and the next change, are always known at a glance. The material is left in the tubes through all the processes up to the first bath in melted paraffine.

The advantages of this method are as follows: there is no loss of time in handling large quantities of small light objects; washing is easy and thorough; there is no danger of injury to the most delicate material; no

labels, except those for identification, are necessary on the separate batches of material; and the series of alcohols and xylols may be used repeatedly, for while there is a continual weakening of each grade in the series, the weakening is proportional throughout the whole series, so that their relation to each other remains practically unchanged.—WINFIELD DUDGEON, *Ewing Christian College, Allahabad, India.*

THE RELATION BETWEEN THE TRANSPIRATION STREAM AND THE ABSORPTION OF SALTS

During the winter of 1908–1909 experiments were conducted at Santiago de las Vegas, Cuba, in order to determine the comparative transpiration of tobacco plants under cheesecloth shade and in the open ground. For this purpose plants were grown in galvanized iron tanks which were set into outer incasing tanks permanently sunk in the ground. Six tanks were placed among the plants of a field of tobacco grown under cheesecloth, and six were set in an adjoining tobacco field not shaded. The quantity of water transpired by the plants in the tanks was determined by daily weighings, the quantity transpired being replaced each day. At maturity the leaves, stems, and roots of each plant were harvested separately, dried, and ground. The ash was determined in water-free samples of the ground material. From the data the total ash of the plants was calculated. The condensed results of the experiment are given in the following table.

PLANTS GROWN IN THE OPEN

No. of plant	Total water-free substance produced, in grams	Total water absorbed, in cc.	Total ash in plants, in grams	Water absorbed per gram of ash
1	209.03	52,066	18.19	2862
3	168.33	42,059	16.74	2512
5	191.91	46,840	20.54	2280
6	187.97	45,418	16.74	2713
7	185.06	46,447	18.86	2463
9	188.20	45,234	18.42	2456
Average	188.42	46,344	18.25	2548

PLANTS GROWN UNDER SHADE

No. of plant	Total water-free substance produced, in grams	Total water absorbed, in cc.	Total ash in plants, in grams	Water absorbed per gram of ash
10.....	211.43	42,122	21.36	1972
12.....	199.08	38,256	21.88	1748
14.....	184.67	36,448	20.15	1809
15.....	172.56	33,965	19.91	1706
16.....	186.80	33,922	21.56	1573
18.....	174.27	32,407	21.61	1500
Average.....	188.14	36,187	21.08	1718

From this table it appears that the total dry substance produced by the plants was about equal in the two sets.

The plants grown in the open absorbed about 28 per cent more water than those grown under shade. The plants which absorbed and transpired the greater quantity of water contained both the smaller percentage and the smaller absolute quantity of ash.

It appears, therefore, that the absorption of salts by roots is independent of the absorption of water, and that the transpiration stream does not exert an accelerating effect on the entrance of salts.—HEINRICH HASSELBRING, *Bureau of Plant Industry, Washington, D.C.*

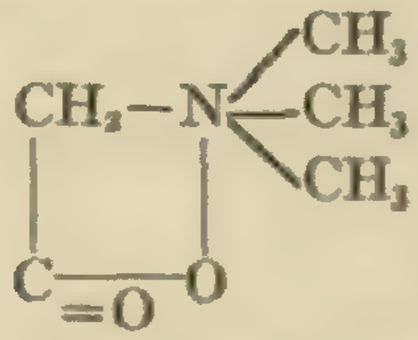
CURRENT LITERATURE

BOOK REVIEWS

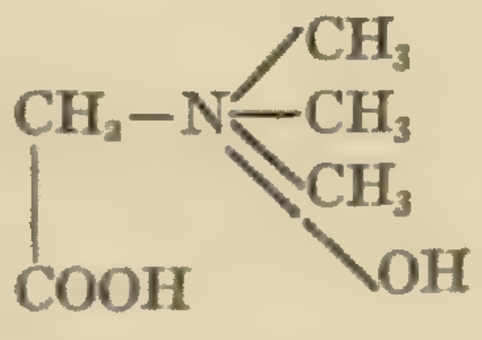
The simple plant bases

A recent book by TRIER[1] will interest students of plant physiology and plant chemistry.

The plant bases are divided into three classes: (1) the high molecular, physiologically active alkaloids peculiar to certain plants; (2) the simple alkaloids without known peculiarities and widely distributed in the plant kingdom; (3) the basic splitting products of the protoplasmic constituents, as proteins, nucleic acids, lecithins, etc. Plant alkaloids are nitrogen-containing bodies which arise in the formation or transformation of protoplasmic substances. By synthetic processes the reaction capacity of their basic H atom is locked up in such a way as to render them unavailable for resynthesis of protoplasmic substances. The primary amines are the simplest alkaloids. They are formed by the breaking up of the carboxyl group of the corresponding amino acids. The simplest case would be the removal of a molecule of CO_2. The amines of nearly all of the amino acids are known. They are seldom found in higher plants as they are at once converted into higher alkaloids either by condensation or by the simple process of methylation, which is a very general process in plants and has the effect of throwing the methylated body out of the field of chemical activity. The betains are completely methylated amines. This relates them directly to the amino acids. They are simple alkaloids and may be further defined as substances in which one assumes an intra-molecular saturation of the amino group with the acid carboxyl group. They cannot replace cholin in the lecithin molecule.



Betain (glycocoll betain)



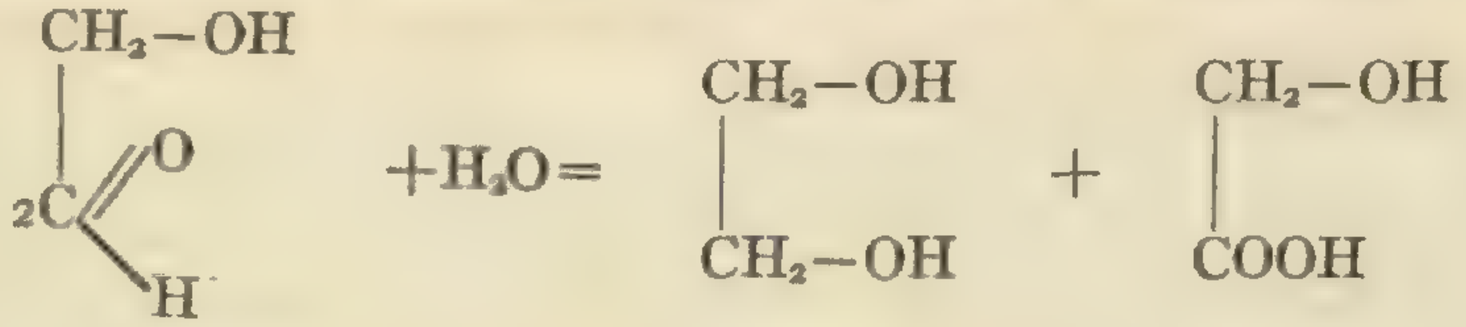
(hydrated form)

Cholin is formed by a methylation process of colamin (aminoethylalcohol), which is the primary amine of serin. This methylation occurs within the lecithin complex; therefore it is not a primary building stone of lecithin but appears only as a hydrolytic product of this substance. Xanthin bases also undergo methylation, as caffein from xanthin.

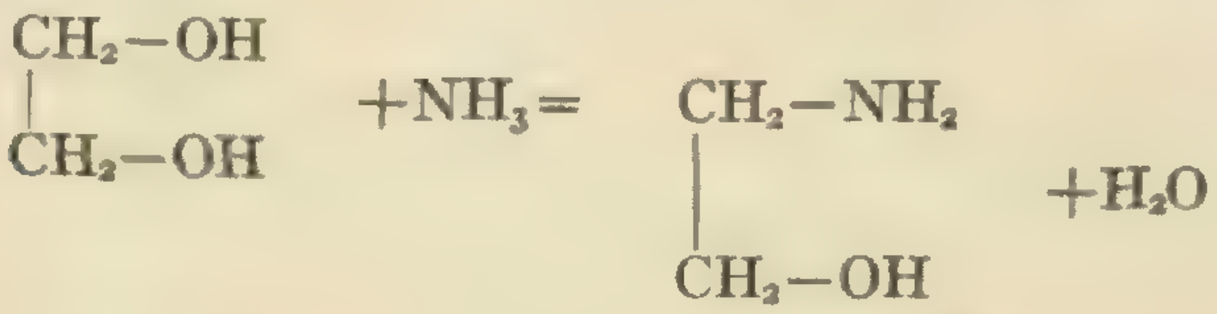
The hypothesis formulated to explain the formation of the simplest amino

[1] TRIER, GEORG, Über einfache Pflanzenbasen und ihre Beziehungen zum Aufbau der Eiweisstoffe und Lecithine. pp. iv+117. Berlin: Gebrüder Borntraeger. 1912.

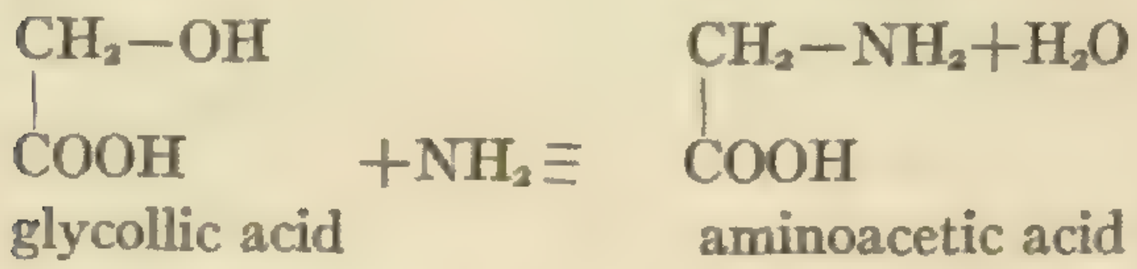
acid includes also an explanation of the primary origin of characteristic building stones of lecithins, since the acids of the proteins and the alcohols of the lecithins arise through one and the same reaction. This would explain STOKLASA's finding that protein and lecithin-formation always run parallel. The formaldehyde, formed by the reduction of CO_2 in green plants, is condensed to glycolaldehyde. By the Cannizzaro reaction, one molecule of glycol and one molecule of glycollic acid arise from two molecules of glycolaldehyde. These products furnish aminoethylalcohol and glycocoll by aminization.



glycolaldehyde glycol glycollic acid



glycol aminoethylalcohol



glycollic acid aminoacetic acid (glycocoll)

This reaction furnishes, therefore, the simplest amino acid, the mother substance of the simplest betain and aminoethylalcohol which may give rise to cholin by methylation as noted above. Analogous with the above reaction a furthur condensation of formaldehyde is postulated for the formation of glycerinaldehyde, and from this may arise glycerin and serin by the reaction of Cannizzaro and by aminization. We now have the glycerin for the formation of fats, lecithin, and other phosphatids. The higher amino acids may be considered derivatives of serin, and alanin a reduction product of the same. The author denies the probability of HCN being an intermediate substance in the primary formation of proteins.

The author extends his hypothesis to the mechanism of methylation. By the reaction of Cannizzaro, formaldehyde can furnish methyl alcohol and formic acid. The methyl alcohol can in turn furnish the alkyl groups for the methylation of metabolic products. Since this scheme is based upon the work of the chlorophyll apparatus, it will not explain the mechanism of the methylations, which frequently occur in animals.

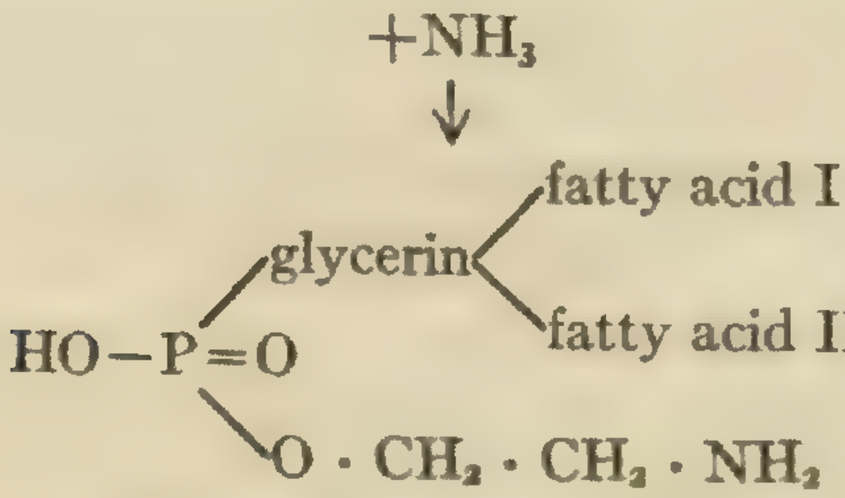
The Cannizzaro reaction is accelerated by the enzyme aldehydase, which has been definitely proven in this case to be a hydrolytic enzyme. In addition to this, if oxygen is present, the alcohols formed at the same time can be oxidized. We have here a case of an enzyme accelerating both hydrolysis and oxidation.

Asparagin, the amide of aspartic acid, has usually been given an important place in studies of protein metabolism in plants. SCHULZE has shown that it must be formed at the cost of amino acids, and according to his investigations it is a secondary product of protein changes and is not a primary building stone of proteins. TRIER cites considerable evidence in support of his contention that aspartic acid, the mother substance of asparagin, is formed from glutamic acid and leucine by oxidation processes. This agrees with the fact that where there is a strong accumulation of asparagin in germination the seed proteins show a high content of glutamic acid.

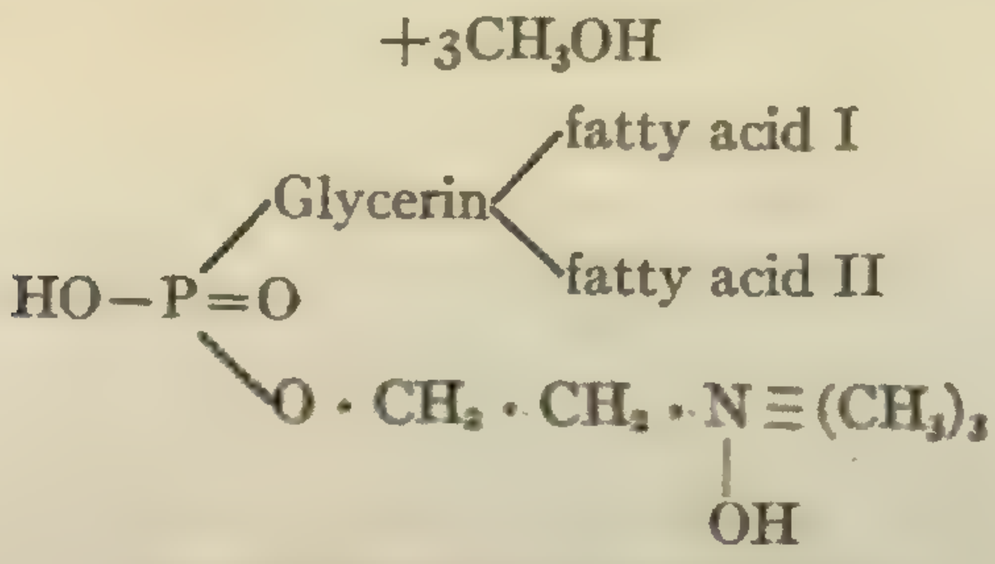
The author's views regarding the part played by the carbohydrates in phosphatid preparations are of great interest, since it is impossible at the present time to fit them into any scheme of lecithin constitution, although TRIER himself has proved that phosphatids from seeds contain reducing substances in chemical combination. He also found that the fatty acids increase and the phosphorus decreases as the content of reducing substances increases. This led the author to think that the regular phosphatid of STRECKER and HOPPE-SEYLER must be accompanied in plants by a substance of the character of animal cerebrosides, that is, substances which furnish, as hydrolytic products, fatty acids and nitrogen bases but no phosphoric acid. The following scheme shows the gradual building up of lecithins according to TRIER:

Formaldehyde → Glycolaldehyde → Glycol → Diglyceridphosphoric acid glycol ester (N free lecithin)

Triglyceride → Diglyceride → Diglyceridphosphoric acid → Diglyceridphosphoric acid glycol ester (N free lecithin)



Diglyceridphosphoric acid-aminoethylester (cholamin lecithin)



Ideal lecithin or cholin lecithin

Those interested in plant physiology and plant chemistry will find this little book of great value on account of the many positive facts stated and because of the critical way in which the author has attempted to organize these facts. Some of the hypotheses may be more sweeping than the facts warrant, but they should serve to stimulate work on these important but difficult problems.—CHAS. O. APPLEMAN.

MINOR NOTICES

Michigan trees.—The first thing that recommends this little manual[2] to the student of trees is its convenient pocket size (5 by 7.5 inches), which makes it more readily useful in the field than more pretentious volumes. A closer examination reveals the fact that it is well illustrated by carefully made drawings of the leaves, flowers, buds, and fruit of each species. The keys seem to have been constructed with more than usual care, and are in duplicate, one based largely upon the leaves, for use during summer; and a second making use of the bud and twig characters as a basis of identification during the winter. In order that the bulletin may appeal to as large an assemblage of readers as possible, the use of technical terms has been reduced to a minimum, and those necessarily employed are fully explained in a glossary. The arrangement of drawings and descriptions of species upon pages facing one another adds to the ease with which the manual may be consulted.—GEO. D. FULLER.

NOTES FOR STUDENTS

Mutations and inheritance in Oenothera.—DAVIS[3] has recently reported a continuation of his studies of *Oenothera*. In previous papers[4] he has described the F_1 and F_2 generations of hybrids between *O. biennis* and *O. grandiflora*. The present account deals with the behavior of F_2 and F_3 generations of the same or similar hybrids. The data presented are discussed (1) from the standpoint of their bearing upon the origin and habit of mutation of *O. Lamarckiana*, and (2) with relation to their possible interpretation by Mendelian principles of inheritance. The latter, if one may judge from the methods employed in these studies, has been incidental to the former. The primary purpose of the investigations has been to determine the possibility of the synthesis through hybridization of a type similar in both taxonomic features and mutating habit

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[2] OTIS, CHARLES H., and BURNS, G. P., Michigan trees. 12mo. pp. xxxii+246. *figs. 120*. Ann Arbor: Univ. Mich. Bull. N.S. **14**: no. 16. 1913.

[3] DAVIS, B. M., The behavior of hybrids between *Oenothera biennis* and *O. grandiflora* in the second and third generations. Amer. Nat. **47**:449–476, 547–571. 1913.

[4] ———, Notes on the behavior of certain hybrids of *Oenothera* in the first generation. Amer. Nat. **44**:108–115. 1910; Some hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. Amer. Nat. **45**:193–233. 1911; Further hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. Amer. Nat. **46**:377–427. 1912.

to *O. Lamarckiana*. The results are summarized briefly as follows: "I have not been able to synthesize by direct crosses, from wild stock so far obtained, any hybrid with all of the characters of *Lamarckiana* in the same plant, although I believe that all of the important taxonomic characters of *Lamarckiana* have been represented in some of my hybrids. . . . . The resemblance of my various hybrids to *Lamarckiana* and the parallelism of their behavior in the F_2 and F_3 to that of *Lamarckiana* give in themselves sufficient reasons, in my opinion, to justify the belief in its hybrid character and to point to the probability that this plant arose as a cross between distinct forms of *Oenothera*. *Lamarckiana* thus would not be representative of a wild species of essentially stable germinal constitution and its mutations are most simply interpreted as the behavior of a hybrid." Prominent among the types that appeared in F_2 and again in F_3 and bred true in a later generation—this behavior constituting the similarity to the mutation habit of *O. Lamarckiana*—were dwarf forms with narrow etiolated foliage, and somewhat similar dwarf forms with normal green foliage. These forms occurred in approximately 10 per cent and 15 per cent of their respective families. Other striking forms were a *semigigas* type with at least 21 chromosomes, and a form similar to *O. elliptica*.

With relation to a possible Mendelian interpretation of his results, DAVIS' most troublesome problems are: (1) "the explanation of the large groups of dwarfs thrown off in the F_2 generations and repeated by certain plants in the F_2," and (2) "the explanation of the well-defined progressive evolution, excluding the dwarfs, exhibited by these same cultures." This "progressive evolution" consisted in the appearance of F_2 families whose flower size ranged from about 1 cm. greater than that of the large-flowered parent (*grandiflora*), to about twice as large as that of the small-flowered parent (*biennis*), and in a corresponding increase in size and amount of crinkling of the leaves. DAVIS doubts the possibility of explaining this advance in flower size on the basis of a recombination of size factors as suggested by the multiple-factor hypothesis, because there was in these families no balancing group with flowers smaller than the small-flowered parent, and he inquires: "What had become in these cultures of the factors responsible for small size?" When it is noted, however, that at least one F_2 family exhibited pronounced "retrogressive evolution" in flower size, little difficulty will be experienced by students of the inheritance of quantitative characters in interpreting DAVIS' results by means of the multiple-factor hypothesis. The obvious suggestion is that the several F_1 plants tested had somewhat different combinations of size factors, that is, that one or both of the parents were heterozygous with respect to a few quantitative factors, a condition which could scarcely be detected under the masking effect of ordinary fluctuation without resort to a careful quantitative study of several lines of progenies of the plants used as parents of any hybrid.

The dwarf plants in certain F_2 and F_3 cultures are also inexplicable to DAVIS on the basis of a recombination of size factors, and with this the reviewer is inclined to agree. The dwarfs are much smaller than the parents, the gap

between them and the parents or the smallest of other F_2 forms is not bridged, and there are no compensating giant forms. It seems more likely that these plants are dwarfs because of some abnormality of function, and that, so far as size factors are concerned, they may be potentially tall. One group in fact is characteristically etiolated. The abnormalities, however, may well be due to a recombination of other genetic factors present in the parents. The fact that the dwarfs have occurred in some cultures in ratios much above 1:15 need occasion little worry at the present stage of the investigations, since students of genetics are coming to look to ratios merely for indications, and to base conclusions rather upon a factorial analysis of the material, worked out by intercrosses of the diverse types of the culture concerned or by back crosses with the parents.

DAVIS' results as a whole are of the greatest importance. It is hoped that he will find the time in the near future to subject his material to statistical and factorial analysis in the same painstaking way that has given the brilliant results secured in his attempted synthesis of *Oenothera Lamarckiana*.

HERIBERT-NILSSON[5] has reported the results of a study of *Oenothera Lamarckiana* and its derivatives. His cultures exhibited numerous heritable differences with regard to such characters as color of leaf veins and leaf blades, breadth of flowers, length of fruits, and height of plants. The appearance of these minor forms in cultures of *Lamarckiana* is regarded as an indication that the species is not a constant one. The characteristics by which these forms are distinguished are the same, in part at least, as those that serve to differentiate the mutations of *Lamarckiana*. This fact suggests to the author that the mutations are the result of new combinations of characters, or factors, present in the parent species.

The mutations that appeared in HERIBERT-NILSSON'S cultures were not identical with those of DE VRIES. Some of them were entirely unlike DE VRIES' mutations, while others were parallel types. Some of the latter resembled *rubrinervis*, *gigas*, *albida*, and *lata*, for instance, in certain respects, but differed from them in others. Certain of the author's mutants combined important characteristics of several of DE VRIES' mutants. One, for instance, exhibited characters of *rubrinervis*, *scintillans*, and *lata*. The forms studied differed principally in quantitative characters, a fact that makes a factorial analysis a matter of extreme difficulty. The behavior of certain qualitative characters, particularly red color of leaf veins, indicated that two or three Mendelian factors might be concerned. Ratios of red-veined to white-veined individuals occurring in the F_3 generation of a giant form approached 3:1, 15:1, and 63:1. A complete factorial analysis of these groups, based upon a study of their progenies, has apparently not as yet been attempted. HERIBERT-NILSSON'S suggestion that giant (*gigas*-like) oenotheras have arisen through

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[5] HERIBERT-NILSSON, H., Die Variabilität der *Oenothera Lamarckiana* und das Problem der mutation. Zeitsch. Ind. Abst. Vererbungs. **8**:89–231. 1912.

the combination of numerous independent size factors is criticized by GATES[6] (1) on the basis of cytological evidence (tetraploid chromosomes) to the contrary, and (2) on the basis of their sudden, discontinuous origin.—R. A. EMERSON.

Araucarians.—Miss HOLDEN[7] has recently described the stems of two fossil plants from eastern Canada, a *Tylodendron* from the south shore of Prince Edward Island and a form which she claims is *Voltzia coburgensis* from the Triassic at Martin's Head, New Brunswick. She has identified her specimens by the casts of the pith, and by the structure as well, and uses her determinations as evidence of the geological horizon of the strata in which they are found. In this connection, she states: "Since *Tylodendron* is characteristic of the Permian, there can be no question that these strata [those of Prince Edward Island] are of that age"; and of *Voltzia:* "Paleobotanical evidence indicates that the Mesozoic strata of New Brunswick are of the same age as those of the eastern United States, and should be correlated with the Lettenkohle or Lower Keuper of Europe." The pith casts of her *Tylodendron* are typical, and she states of the ligneous structure: "It agrees exactly with that described by DAWSON from Mr. BAIN'S specimen as *Tylodendron Baini*, and with that described by POTONIÉ as *Araucarioxylon rhodeanum* Goepp."

In discussing the evidence for and against the generally accepted view of the araucarian affinity of *Tylodendron*, a view, however, from which Miss HOLDEN dissents, she agrees with POTONIÉ that "the nodal swellings and instanding protoxylem strands causing the ridges and furrows of the pith casts are identical with similar structures in *Araucaria* and *Agathis*," but states that instanding protoxylem strands are common to all living conifers. She admits that the medullary rays are typically araucarian, the rays uniseriate, rarely over 10 cells high, and composed of thin-walled cells, but she says that all conifers have uniseriate rays. Neither of her arguments, however, precludes the araucarian connection. Of the tracheary pitting, she says: "Its closely compressed and alternating pits clearly affiliate it with *Araucarioxylon* Krauss," but she considers that this does not indicate araucarian affinity, since "closely compressed and alternating pitting is not the primitive condition for the Araucarineae." This statement is made on the authority of Professor JEFFREY'S[8] recent work. While both of these articles were in press, however, the writer[9] advanced evidence

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[6] GATES, R. R., Tetraploid mutants and chromosome mechanisms. Biol. Centralbl. **33**:92–99, 113–150. 1913.

[7] HOLDEN, Miss R., Some fossil plants from eastern Canada. Ann. Botany **27**: 243–255. 1913.

[8] JEFFREY, E. C., The history, comparative anatomy, and evolution of the *Araucarioxylon* type. Proc. Amer. Acad. **48**:531–571. 1912.

[9] THOMSON, R. B., On the comparative anatomy and affinities of the Araucarineae. Phil. Trans. Roy. Soc. B **204**:1–50. 1913.

to show that the reverse is true. The pitting, for example, in the *cone axis* of *Araucaria Bidwillii* may be as much as 5-seriate, the pits alternating and extending from end to end of the tracheid. In this and in other primitive regions as well, the mouth of the pit is elliptical, a vestige of the more ancient scalariform condition, a condition which is retained even longer where the medullary ray touches the tracheid. No torus is present in these regions too, although this is well developed in the whole pine alliance. Again, Miss HOLDEN says: "Impressions present more evidence for merging *Tylodendron* with the araucarians. Several varieties of leafy branches, known as *Walchia*, and definitely associated with *Tylodendron* pith casts, have been described, all bearing a close resemblance to different species of *Araucaria*. Of their fructifications little is known, further than that, as shown by ZEILLER, the scales of the female cone bear single seeds, another araucarian feature." She presents nothing in opposition to the above statement, but in concluding the paragraph says: "If these criteria are reliable, the presence of *Tylodendron* in the Permian strata bears out the orthodox view that the Araucarineae are the oldest living family of the Coniferales." Since Miss HOLDEN has not invalidated any of these criteria, the case must hold for the araucarian connection. She evidently fears to draw this conclusion on account of the temerity of the advocates of araucarian ancestry of the conifers, for her final point is that "there are woods of the *Tylodendron* type extending as far back as the Culm, yet no advocate of the antiquity of the araucarian line would suggest that it extends as far back as that."

On the other hand, Miss HOLDEN considers that her more recent form *Voltzia coburgensis* from the Triassic is an araucarian, but one derived from the Abietineae. She accepts the conclusion as to the araucarian affinity of *Voltzia*, though she has rejected this conclusion in the case of *Tylodendron* which has one point more in its favor. The character of the rays, etc., of *Voltzia* is described as distinctly araucarian, just as in *Tylodendron*. The leaf trace is single at the pith, but forks during its passage through the wood, a similar condition, as Miss HOLDEN states, to that in *Agathis*. She has previously (p. 246) drawn attention to "the araucarian single trace" in *Tylodendron*. Of the pits in *Voltzia*, she says that they are "always uniseriate and usually scattered . . . . rarely are they so closely compressed as to be flattened and angular. While they are as distant as the pits of the Abietineae and Taxodineae, they are never, as is the rule in these groups, separated by so-called bars of Sanio." This pitting is the one point of difference from *Tylodendron*, where the pits are typically of the *Araucarioxylon* type, and the one point more in favor of the araucarian connection if the latter.

The anatomical evidence of abietinean affinity of *Voltzia* is said to be "the scattered position of the pits." Why this is distinctive of the Abietineae is not clear. She herself states (see the quotation in the preceding paragraph) that it is found in the Taxodineae, and it occurs in other conifers as well. Nor is it evident why the cone is abietineous, as Miss HOLDEN states. She refers to nine authorities, only one of which agrees that it is abietineous. Three refer

it to the Cupressineae and four put it with the Taxodineae, as the original describer, BRONGNIART, also did. No reasons are given for Miss HOLDEN'S choice.

After discussing the combination of araucarian and abietinean characteristics in *Voltzia*, she speaks of other forms showing similar combinations, and says: "Dr. JEFFREY . . . . appears to have demonstrated that the Abietineae are older, and that it is the Araucarineae which become progressively more and more like the Abietineae in successively older geological formations." Certainly this is not the case in the two forms she describes, even disregarding the evidence from the cone in both cases which is known in impression only. When, however, the cone impressions are given equal importance in each case, the foregoing conclusion is further at variance with the facts. Nor is the case improved by including the other transitional forms, which are considered important by the Harvard school, *Woodworthia* of the Triassic and *Araucariopitys* of the Cretaceous, since the former is practically an araucarian and the latter an abietinean. So far then as the evidence from the transitional forms stands, the reverse of the conclusion attributed to Professor JEFFREY is the fact. It is the Abietineae which are more like the Araucarineae in the older geological formations. When this evidence is taken in connection with the fact that no true Abietineae have been described from the strata preceding the Triassic, the historical evidence is seen to be wholly adverse to the Abietineae.

Miss HOLDEN'S own work then, far from supporting the abietinean ancestry of the Araucarineae, is directly opposed to it. Had the full evidence of the character of the ancestral pitting in the araucarians been before her, she would probably have escaped the pervasive influence of this theory.—R. B. THOMSON.

Pityoxylon.—One of Miss HOLDEN'S[10] three new species of Pityoxyla from the Middle Cretaceous of Cliffwood, N.J., is "probably the earliest form with all the characters of a modern hard pine, yet retaining certain ancestral features, as the association of primary and fascicular leaves." She has appropriately designated this form *Pinus protoscleropitys*. Its occurrence in the Middle Cretaceous is regarded as "an argument for the great geological antiquity of the pines as such." Her *Pityoxylon foliosum* is "possibly the wood of *Prepinus*, with all its leaves borne directly on the main axis," and combining the characteristics of both hard and soft pines. The third form, *Pityoxylon anomalum*, has much the same type of wood structure as the second, but has "all its leaves borne on short shoots."

The spur shoots are described as large in both forms, "much larger than those of living pines," but unbranched, as in modern pines, and thus unlike those of *Gingko*, or *Woodworthia* from the Triassic whose spurs were also large. The large size of the spurs in the old fossil forms is evidence that the spur was ancestrally a branch.

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[10] HOLDEN, Miss R., Cretaceous Pityoxyla from Cliffwood, New Jersey. Proc. Amer. Acad. **48**:609–623. 1913.

The resin canals, both horizontal and vertical, are said to be tylosed generally. Since there is no evidence presented that they were ever open, this is probably not a true tylosed condition, but rather the solid condition of the ancestral forms, a condition which is very prevalent in the older fossils. It is to be noted also that in the two forms which Miss HOLDEN considers more primitive than *P. protoscleropitys*, resin canals are said to be filled with *thick-walled* cells. In one of them, *P. foliosum*, where the resin ducts are very numerous, they are frequently in tangential groups of three or four. The fact that tangential series of resin canals can be revived in the living pines by injury, and that such traumatic resin canals are usually solid is in agreement with the fossil condition, and indicates that the resin ducts of the pines were ancestrally of this type.

In all three forms, the pits are usually uniseriate and scattered. In *P. protoscleropitys*, Miss HOLDEN speaks of the terminal pits of the tracheids as being sometimes "closely approximated and flattened by mutual contact." She has not described this pitting in the other two. No bars of Sanio have been observed except in the former, the most specialized form. The ray pitting of its tracheids, too, shows a tendency toward the formation of "Grosseiporen," while theirs is piciform, the more primitive condition. Tangential pitting of the summer wood is absent in *P. protoscleropitys*, but present in the others. She says that this confirms "the conclusions of JEFFREY and CHRYSLER that tangential pitting is a primitive feature now lost in the more highly specialized hard pines." She has evidently overlooked STRASBURGER'S previous statement of this conclusion (Hist. Beiträge 3:9. 1891), as did JEFFREY and CHRYSLER themselves.

Miss HOLDEN'S *P. protoscleropitys* has the sculptured ray tracheids of a hard pine, while the other two forms have no ray tracheids. She has looked in the former for verification of the mode of origin of ray tracheids proposed by THOMPSON,[11] from vertically elongated tracheary elements, but on finding none disparages the correctness of THOMPSON'S work, stating that it is "unlikely that this hypothesis is correct." She has evidently not understood the problem, for the form in which she looked for evidence is, by her own statement, a specialized one in this very feature. Again, she was dealing only with the *stem*, while THOMPSON worked chiefly with the more conservative organ, the *root*. Moreover, the recent investigations of CHRYSLER,[12] who has thoroughly worked over the ground from the standpoint of the phloem, have confirmed THOMPSON'S conclusion. He found the evidence in the root so much clearer than in the stem that he says he soon discontinued the study of the latter.—R. B. THOMSON.

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[11] THOMPSON, W. P., The origin of ray tracheids in the Coniferae. BOT. GAZ. 50:101–116. 1910.

[12] CHRYSLER, M. A., On the origin of erect cells in the phloem of the Abietineae. BOT. GAZ. 56:36–50. 1913.

A new interpretation of mitosis.—In 1911 DEHORNE published two papers[13] setting forth a new interpretation for the phenomena of somatic and heterotypic mitosis in animals and plants. According to this author, the units usually called chromosomes are in all stages of all divisions associated in pairs, each pair having the value of a longitudinally split single chromosome. At metaphase they are not divided along this split, but are simply separated into two groups which pass toward opposite poles. During anaphase the members of each pair separate somewhat from each other and become secondarily split. After persisting through the resting stages as interlaced spiral threads, these two double structures are finally separated at the next metaphase. Thus the line of separation at any metaphase is determined during the second preceding anaphase. The diakinetic pairs are in like manner regarded as longitudinally split somatic chromosomes. At the first maturation division one-half of these pairs goes to each pole, bringing about a reduction. During anaphase each member is longitudinally split as in the somatic mitoses. At the second division instead of separating into their longitudinal halves, they are distributed in two groups of double rods. According to this interpretation the haploid number of chromosomes in *Lilium* should be regarded as 6 and the diploid number as 12, rather than 12 and 24.

GRÉGOIRE,[14] in a very detailed description of the metaphase and anaphase in *Galtonia*, *Trillium*, and *Allium*, demonstrates clearly that in every case a dicentric separation of the halves of each chromosome occurs, and that there is no such pairing as DEHORNE has described. In a second short note[15] he shows, after a careful study of *Lilium*, that the phenomena of maturation follow the heterohomeotypic scheme previously described by him, and contradict in all points the conclusions of DEHORNE. What is true of *Lilium* is held by GRÉGOIRE to be generally true of all higher plants and many animals. These results, together with those of MUCKERMANN[16] on *Salamandra* and other forms, are conclusive in showing that the interpretation of DEHORNE is wholly false.—L. W. SHARP.

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[13] DEHORNE, A., Recherches sur la division de la cellule. I. Le duplicisme constant du chromosome somatique chez *Salamandra maculosa* Lour. et chez *Allium Cepa* L. Archiv f. Zellforschung **6**:613–639. *pls. 35, 36. figs. 2.* 1911; Recherches sur la division de la cellule. II. Homéotypie et hétérotypie chez les Annélides polychètes et les Trémotodes. Arch. Zool. Exp. et Gén. **9**: 1911.

[14] GRÉGOIRE, V., Les phénomènes de la métaphase et de l'anaphase dans la caryocinèse somatique. À propos d'une interprétation nouvelle. Annales Soc. Sci. Bruxelles **34**:pp. 36. *pl. 1.* 1912.

[15] ———, La vérité du schéma hétérohoméotypique. Compt. Rend. **155**:1098–1100. 1912.

[16] MUCKERMANN, H., Zur Anordnung, Trennung, und Polwanderung der Chromosomen in der Metaphase und Anaphase der somatischen Karyokinese bei Urodelen. La Cellule **28**:233–252. *pls. 2.* 1912.

Soil moisture measurement.—The water content of the soil has long been recognized as the most important edaphic factor in limiting the occurrence and permanence of plant associations, but it has always been difficult to measure such a factor in terms that could be related to plant production. CRUMP,[17] in his studies of the vegetation of peat soils, has devised a method of expressing the relative humidity of these soils in such a manner that a definite index of water as an ecological factor is obtained. This index he has termed the "coefficient of humidity," and it seems, for the habitats studied, to be a constant whose value may be determined for any given plant association. In obtaining this constant, the amount of water present in any soil is expressed in terms of percentage of the dry weight at 15° C., and the humus-content being determined in the usual way by combustion, the ratio of the water-content is obtained in terms of the humus-content as follows:

$$\frac{\text{water-content}}{\text{humus-content}} = \text{coefficient of humidity}$$

This coefficient is shown to vary directly with the amount of water available for the use of the vegetation of a habitat, and the investigator believes it to be a true integration of the relative humidity of the soil of different areas. He admits, however, that his methods will not apply to sandy soils with small humus-content, and probably not to many clays, although he has devised a correction which permits it to be used for sub-peats containing large amounts of sand.

Applying this unit of measurement to certain moor plant associations, he finds[18] that the mean coefficients of humidity for the *Eriophorum* moor, the *Calluna* moor, and the *Molinia* moor of the Southern Pennines to be respectively 6, 3·3, and 2; and thus he is able to institute a direct comparison between the water conditions of these associations and others in the same formation.

It would seem that as the result of these investigations the ecologist has been given a most important method of expressing soil moisture, far in advance of anything before available, and it is to be hoped that it will be found to be applicable to a great variety of soils.—GEO. D. FULLER.

Chromosomes in Allium.—In the nuclei of *Allium Cepa* BONNEVIE[19] has described a large chromatin knot from which the chromatin threads radiate. In the presynaptic stages in the pollen mother cells these threads become paired. From a comparison with the origin and behavior of similar radial threads in

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[17] CRUMP, W. B., The coefficient of humidity: a new method of expressing the soil moisture. New Phytol. **12**:125–147. 1913.

[18] CRUMP, W. B., Notes on water content and the wilting point. Jour. Ecol. **1**:96–100. 1913.

[19] BONNEVIE, K., Chromosomenstudien. III. Chromatinreifung in *Allium Cepa* (3). Arch. f. Zellforschung **6**:190–253. *pls. 10–13*. 1911.

the somatic divisions, the conclusion is drawn that this process represents a side-by-side conjugation of somatic chromosomes, which are separated at the first maturation division.

MOTTIER and NOTHNAGEL,[20] after a study of the pollen mother cells of *Allium cernuum*, come to very different conclusions regarding the conjugation process. These stand in agreement with the earlier accounts of MOTTIER, and may be summarized as follows. Synapsis is a real contraction of the linin net with its chromatin granules. The thick spirem which emerges therefrom shows only an occasional temporary split in some parts. Nothing is found to indicate a union of two spirems in the prophases. During a second contraction the spirem is thrown into loops, and cross-segmentation occurs. The bivalent chromosomes so formed are regarded as consisting of two somatic chromosomes previously arranged end to end in the spirem. The members of each bivalent separate at the first division and during anaphase become longitudinally split in preparation for the second. At telophase there is formed an interrupted spirem, but there is present no chromatin knot such as BONNEVIE has described and which MOTTIER and NOTHNAGEL believe to be due to improper fixation.

The above works recall the earlier researches of BERGHS[21] and of GRÉGOIRE[22] on *Allium fistulosum*, in which they found the bivalent chromosomes arising through a union of two spirems in the presynaptic or synaptic stages, as BONNEVIE later found in *Allium Cepa*. The figures given by these writers to illustrate these critical stages form a much more complete series than those accompanying the contribution of MOTTIER and NOTHNAGEL. It is hardly probable that the disagreement between these accounts is due entirely to the fact that different species of *Allium* were used.—L. W. SHARP.

Cytology of mutants.—Some of the cytological aspects of the *Oenothera* question are summarized by GATES[23] in a discussion of tetraploid mutants. *O. gigas*, a tetraploid form, in all probability arises through the apogamous development of an unreduced megaspore mother cell with 28 chromosomes ($4x$), such a cell having been seen by GEERTS. Triploid mutants seem to be due to the union of a diploid with a haploid germ cell (STOMPS, Miss LUTZ). In some plants the mutational changes are not confined to the meiotic divisions, but at

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[20] MOTTIER, D. M., and NOTHNAGEL, M., The development and behavior of the chromosomes in the first or heterotypic mitosis of the pollen mother cells of *Allium cernuum* Roth. Bull. Torr. Bot. Club **40**:555–565. *pls. 23, 24.* 1913.

[21] BERGHS, J., La formation des chromosomes hétérotypiques dans la sporogénèse végétale. II. Depuís la sporogonie jusqu'au spirèm définitif dans la microsporogénèse de l'*Allium fistulosum*. La Cellule **21**:383–394. *pl. 1.* 1904.

[22] GRÉGOIRE, V., La formation des gemini hétérotypiques dans les végétaux. La Cellule **24**:369–420. *pls. 2.* 1907.

[23] GATES, R. R., Tetraploid mutants and chromosome mechanisms. Biol. Centralbl. **33**:93–99, 113–150. *figs. 7.* 1913.

many different stages irregularities in chromosome distribution may occur in a variety of ways. GATES believes several characters of *O. gigas* cited by DE VRIES as occurring independently of chromosome doubling are the result of the tetraploid condition with its larger cells and nuclei. Many such differences are attributed to causes fundamentally quantitative. The interpretation of NILSSON, that *O. gigas* originates by the accumulation of factors for size, is held by GATES to be contradicted by the cytological facts and by the sudden origin of giant types with their subsequent wide variation. Although some *Oenothera* characters are Mendelian in their behavior after they appear, Mendelian combinations in NILSSON'S sense are inadequate to account for their first appearance.—L. W. SHARP.

A heterosporous fern.—LIGNIER[24] has published a new genus (*Mittagia*) from the Lower Westphalian strata, which is the first heterosporous fossil fern to be described. That such a group did occur is, of course, postulated by the existence of the seed ferns, but this is the first demonstration of its presence. LIGNIER, too, has sounded a note of warning by his discovery. He was at first inclined to consider that his sections were of a pteridosperm, *Lagenostoma Lomaxi*, so similar in structure are the outer tissues of the sporangia in the two forms. When he found four megaspores to a sporangium, a stomium present, and the sporangia arranged in a sorus, he knew that he had something different. His sporangium, however, he considers did not dehisce, and so, like *Lepidocarpon*, is a stage toward the seed habit. His conclusion that the sporangia belonged to a fern is based chiefly on their structural resemblance to *Lagenostoma*, and on their arrangement in a sorus. He has further distinguished them from the lycopod and equisetum lines, from *Lepidocarpon*, *Miadesmia*, *Selaginella*, heterosporous calamites, etc. LIGNIER'S intensive study of the small amount of material at his disposal and his logical deductions are exceedingly interesting and valuable.—R. B. THOMSON.

Evaporation in Skokie Marsh.—Using the Livingston atmometer, SHERFF[25] measured the evaporating power of the air in a marsh habitat near the city of Chicago during the summer of 1911. The average daily rate of evaporation for the lowest stratum of vegetation was 3 cc. for the *Typha* association, 4.27 cc. for the reed swamp, 4.5 cc. for the swamp meadow, and 7.9 cc. for the swamp forest of *Quercus bicolor* and *Fraxinus americana*. This forest is normally antecedent to a truly mesophytic forest such as that found by the reviewer to have an average daily rate of 8.1 cc.[26] During September and October of the

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[24] LIGNIER, O., Un noveau sporange séminiforme. Mém. Soc. Linn. Normandie **24**:49–65. 1913.

[25] SHERFF, E. E., Evaporation conditions at Skokie Marsh. Plant World **16**:154–160. 1912.

[26] BOT. GAZ. **52**:193–208. 1911.

same season SHERFF also obtained data upon the evaporation rates in different strata of the marsh vegetation, showing the evaporating power of the air to be 300 per cent greater in the top stratum of the *Phragmites* association than in the lowest, while the difference became three times as great in the *Typha* association. These results confirm those of YAPP[27] for a sedge vegetation and those of the reviewer[28] for the beech-maple forests, warranting the conclusion that plants may grow in proximity with each other and yet, vegetating in different horizontal strata, be subject to widely different growth conditions.—GEO. D. FULLER.

History and origin of monocotyledons.—HORWOOD[29] has done useful service in bringing together, in convenient form, the evidence of fossil monocotyledons. The record of each family is recited, and the summary shows that the first authentic specimens are from the Cretaceous, and that in the Tertiary or Post-Tertiary 24 families out of about 30 are represented. In dealing with the origin of monocotyledons, HORWOOD gives a synopsis of most of the views that have been advanced and reaches the following general conclusion: that the monocotyledons and dicotyledons are divergent series from a common ancestor; that among the dicotyledons there has been "progression and differentiation," while among the monocotyledons there has been "retrogression and even some reduction from a common ancestor of the primitive angiospermic type." This primitive type, by the way, "resembled an alismaceous or liliaceous type, on the one hand, and a ranalian type on the other," and in the background of this primitive stock the author sees the Cycadafilicales and Bennettitales. The mass of facts brought together will be very useful, even if the conclusions are not convincing.—J. M. C.

Fourth International Botanical Congress.—The first circular of the International Botanical Congress of 1915 has been issued. The sessions will be held in London from May 22 to May 29. Membership is secured by subscribing to the regulations of the congress and by the payment of a subscription of 15 shillings. Ladies accompanying members may attend the meetings and excursions of the congress on payment of 10 shillings each. The presidents of the organizing committee are Professor F. O. BOWER, Sir DAVID PRAIN, and Professor A. C. SEWARD. The general secretary is DR. A. B. RENDLE, British Museum, Cromwell Road, London, S.W., to whom applications may be made.

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[27] YAPP, R. H., On stratification in the vegetation of a marsh, and its relations to evaporation and temperature. Ann. Botany **23**:275–320. 1909.

[28] BOT. GAZ. **54**:424–426. 1912.

[29] HORWOOD, A. R., The past history of monocotyledons, with remarks on their origin. Scottish Bot. Review **1**:164–180, 216–234. *pls. 1–4.* 1912.

Volume LVII Number 2

THE BOTANICAL GAZETTE

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FEBRUARY 1914

Studies on the Reactions of Pilobolus to Light Stimuli — Hally D. M. Jolivette

Morphology of Thismia americana — Norma E. Pfeiffer

Concerning the Presence of Diastase in Certain Red Algae — E. T. Bartholomew

The Male Gametophyte of Abies — A. H. Hutchinson

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THE
BOTANICAL GAZETTE
FEBRUARY 1914

STUDIES ON THE REACTIONS OF PILOBOLUS TO LIGHT STIMULI

HALLY D. M. JOLIVETTE

(WITH TWELVE FIGURES)

The present investigation concerns itself for the most part with the problems of simultaneous stimulation. It was undertaken with a view to settling some of the problems suggested in a former paper on the light reactions of *Pilobolus* by Miss ALLEN and myself (1). Some of the objections inherent in the methods used in the earlier work were eliminated in the experiments here reported, and a study was also made of the reaction of single sporangiophores toward the light.

The work was begun under Dr. R. A. HARPER at the University of Wisconsin and completed under Dr. G. J. PEIRCE of Leland Stanford Junior University. I wish to acknowledge my indebtedness for their criticisms and suggestions. I wish also to express my gratitude to Dr. D. H. CAMPBELL for his interest in my work and for his courtesy in extending all the privileges of his laboratory. I thank Miss RUTH F. ALLEN, who began the study of the reactions of a single sporangiophore of *Pilobolus* toward the light and furnished the data taken on the evenings of May 18 and 19, 1910.

The present study of simultaneous stimulation by light is on the question of directive influence, and the literature discussed will concern that phase of light effect. The early work was carried on with light and gravity as the stimuli. NOLL (4) in 1892 pub-

lished his theory of "heterogene induction." He reviewed the earlier authors on the subject and described their conflicting results. According to NOLL's theory, if an organism is subjected to two stimuli, one gives impetus to the other which carries out the reaction. One stimulus only is responded to. There is no resultant reaction toward the two stimuli. But when light and gravity work together, there is a change of geotonus due to light.

Recently GUTTENBERG (2) studied the simultaneous effect of light and gravity, using seedlings of *Avena sativa*, *Brassica Napus*, *Agrostemma Githago*, and *Helianthus*. He used what he called the compensation method. He tried different light intensities. With the higher intensities the reaction was toward the light alone. By gradually decreasing the intensities, GUTTENBERG found a certain light strength which just equaled that of gravity, and he obtained a resultant reaction between the two. A little weaker or a little stronger light gave a resultant reaction, varying according to the intensity of the light. GUTTENBERG considers this as evidence against NOLL's theory of "heterogene induction."

RICHTER (5), working along the same lines as GUTTENBERG, came to quite different conclusions. For his experiments RICHTER used *Avena sativa*, *Vicia sativa*, *Vicia villosa*, *Brassica Napus*, and *Helianthus*. He followed GUTTENBERG'S method and in each case carried on a set of experiments in pure air and a similar one in impure air. He concludes that GUTTENBERG did not establish a resultant reaction between the effect of light and gravity by means of his compensation method, but that the latter's results were influenced by the impure air in which the experiments were performed. GUTTENBERG (3) followed this by a second paper in which he still maintained his former views. In this he repeated his own experiments, taking precautions to work under pure air conditions.

The experiments on simultaneous stimulation reported in this paper were performed with stimuli of the same kind, that is, they were light stimuli only. Before entering into a description of the work of simultaneous stimulation of *Pilobolus*, an account of some observations made on the reaction of a single sporangiophore of *Pilobolus* will be given.

Study of the reactions of the individual sporangiophore to a single light

In the earlier experiments (2), and also in those on simultaneous light stimulation in this paper, I was concerned with a large number of sporangiophores and with the net result of the reaction. This set of experiments was inaugurated for the purpose of following in detail the stages in the reaction of the individual sporangiophore toward light. The horizontal microscope was employed for this purpose.

A culture of *Pilobolus* in a 5-cm. flower pot was used. The pot was supported in an upright position. A thin glass Petri dish, measuring 5 cm. in diameter and 4 cm. in height, was placed over the top of the flower pot to keep the culture from drying. A 16-c.-p. carbon filament incandescent light was placed at a distance of 30 cm. from the culture, with the central point of the filaments 5 cm. above the level of the surface of the culture. The experiments were performed in the dark room and no other light had access to the culture. A horizontal microscope was placed with the tube on a level with the surface of the culture and at right angles to the direction of the light rays reaching the culture, so that any bending toward the light could be observed. A micrometer scale was placed in the ocular of the microscope in order to measure the change of position of the sporangiophore. In favorable cases several sporangiophores could be observed in the field of the microscope.

The first culture used in these experiments was put in place at 7:15 P.M. The sporangiophore had been exposed to the afternoon light and had grown straight out toward it, making an angle of 45° with the vertical. The culture was placed with the sporangiophores leaning away from the light, so that the angle between the light rays and the sporangiophore was about 135°. At the time when the experiment was set up, the young sporangiophores showed no signs of the sporangial swelling or vesicular bulb.

Two sporangiophores were observed during a period of 3 hrs. on the evening of May 18, 1910, and sketches were made at intervals during the reaction. The exact time when the reaction of the

first sporangiophore became perceptible was not determined. The reaction was distinctly visible at 7:45, 30 min. after it was exposed to the light. So far as could be seen, the curvature began at the tip of the sporangiophore, the tip bending as it grew. The tip distinctly started to curve upward at 7:45. This curve was somewhat more pronounced at 7:50. The tip was vertical, having moved through an angle of 45°. The radius of curvature was short. At 8:40 the tip had grown so that it was no longer vertical, but made a smaller angle with the incident light rays. At 9:30 the tip had grown around so that it pointed in the direction of the light. It had curved about 135° since the beginning of the observation. The curvature took place as the growth occurred; the curved end of the sporangiophore at this period was a well rounded hook. From

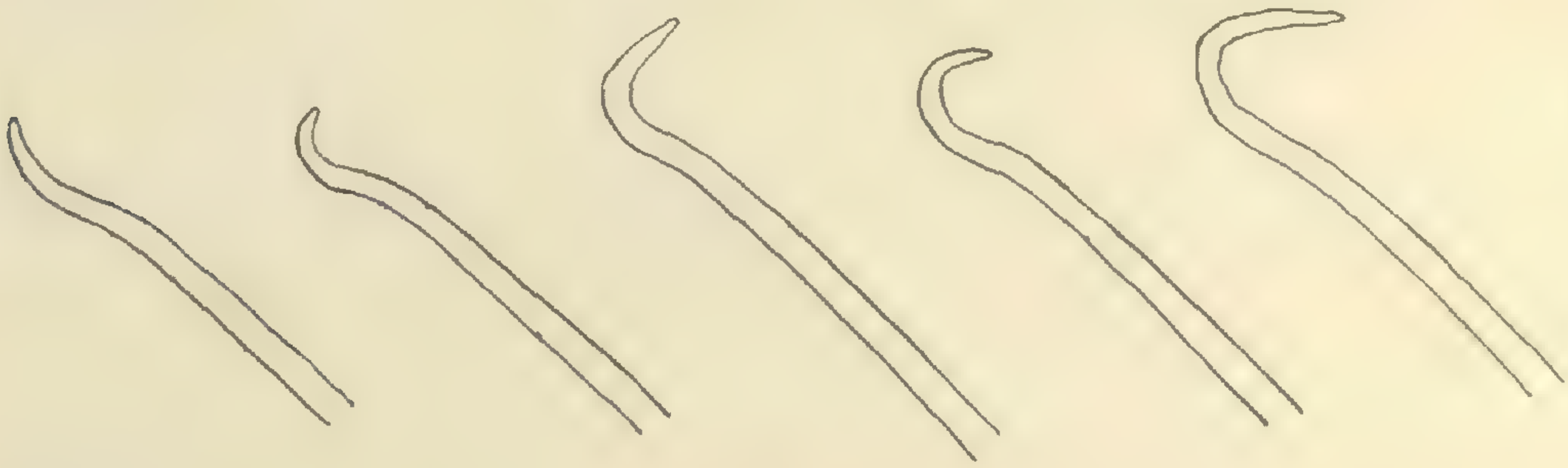

FIG. 1

this time on the tip grew straight toward the light. The last observation on this sporangiophore was made at 10 P.M. and showed a pronounced growth in the direction of the light.

Fig. 1 shows the stages that were sketched. The arrow indicates the direction of the light. The bending in this case had taken place always at the tip, the growing point of the sporangiophore. The older basal portion of the sporangiophore appeared to maintain the form and position which it had at the beginning of the experiment. If there was any change, it was so slight as not to be detected with the microscope.

The behavior of another sporangiophore under observation at the same time was as follows. The reaction was somewhat longer, no sign of curvature being noticeable until 8:00 P.M., 45 min. after the beginning of the experiment. The bending progressed slowly. At 8:40 the tip had curved through 45°, the curve being gradual.

At 9:15 the tip seemed to have stopped bending and to have grown straight. The angle made with the light was 60°. From 9:15 to 9:50 the increase in length was slight, and the older portion of the curve seemed to have become slightly more bent. At 10:15 the terminal sporangial swelling was well defined and the limits of the vesicular bulb could be discerned. The direction of the tip remained unaltered and formed an angle of 60° with the incident light rays (fig. 2).

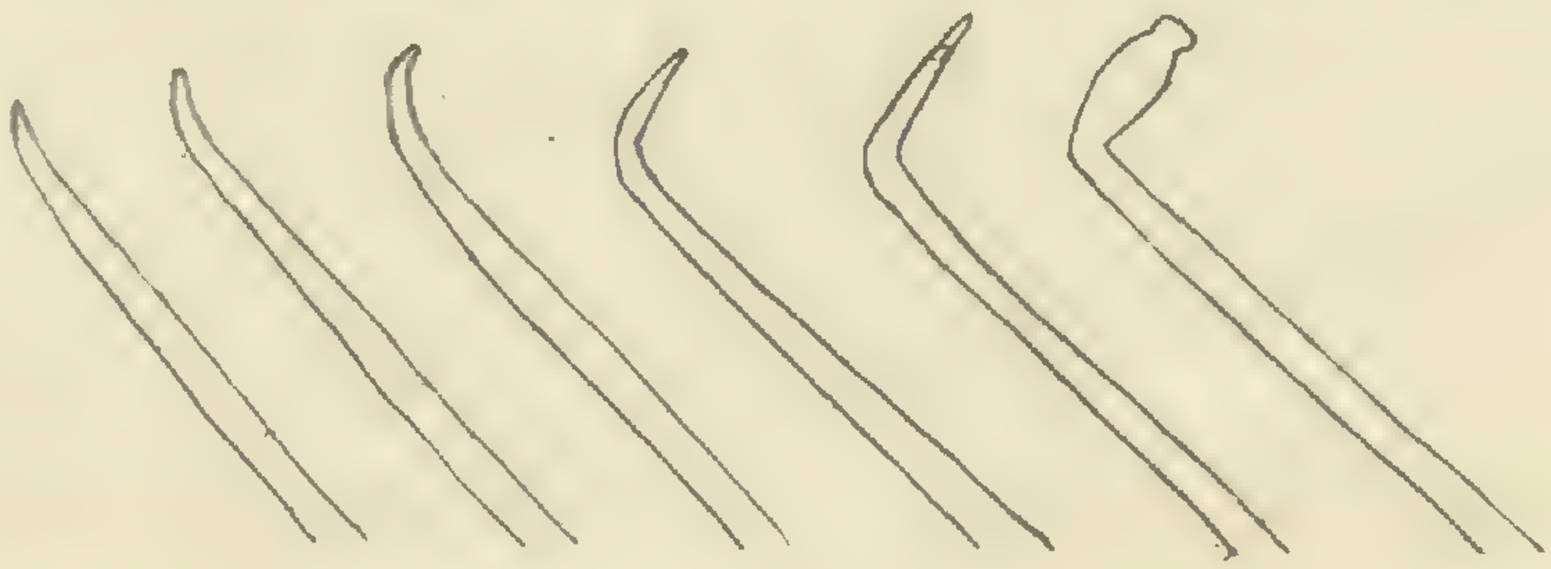

FIG. 2

A group of sporangiophores on the same culture as the two described above and subjected to the same stimulation from 7:15 P.M. until 9:55 were observed at 9:55. Three of them were still turned in the direction from which the afternoon light had come and away from the light used in the experiment. Apparently there was no response toward the light stimulus. In these three cases sporangium-formation had begun. The fourth sporangiophore had been subjected to the same conditions. The tip was curved through 135° and proceeded to grow in the direction of the light. The tip was still slender and pointed. The difference in length between this one and the other three was noteworthy. It exceeded the other three by the length of the portion beyond the bend where it turned toward the light. The sketches of the four sporangiophores as they were at 9:55 are shown in fig. 3.

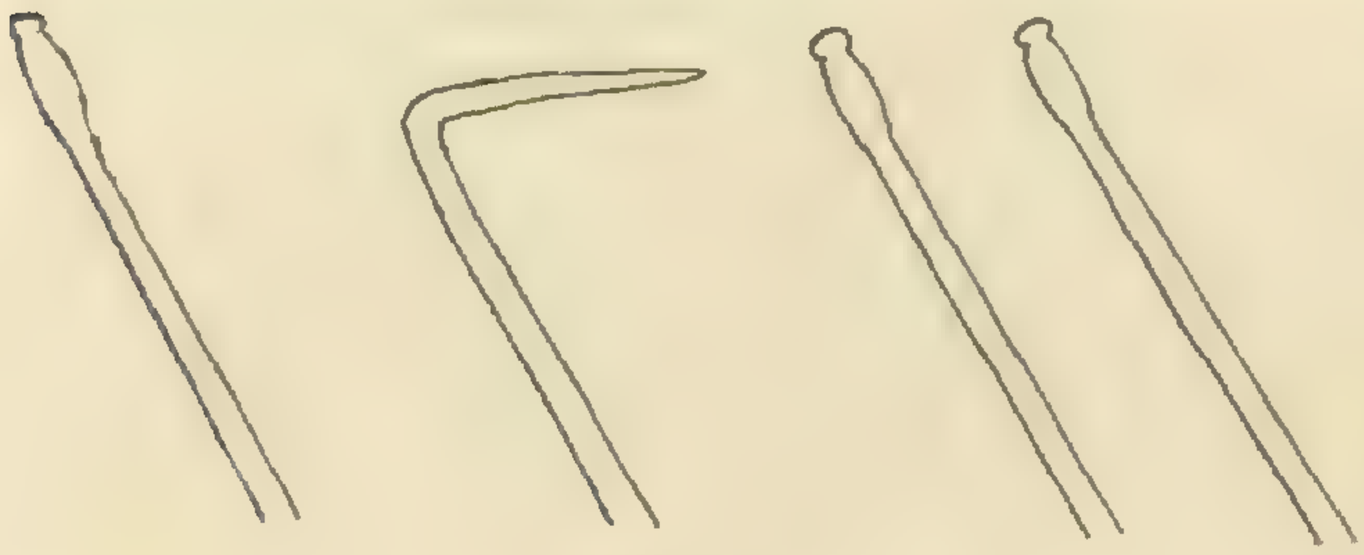

FIG. 3

At 8:15, May 19, 1910, an older culture was used. Sporangium-formation had started when the observations were begun. At the beginning of the experiment the five that were chosen for study were at different stages, the youngest showing the sporangium as a

small yellow knob, and the oldest having its sporangium full grown and turning black. They were all pointed in the direction of the afternoon light, making an angle of 135° with the incident electrical light rays. The five were watched closely from 8:15 to 9:55 P.M., the position of the tip of each being observed and recorded every 5 min. During this time their development continued normally, the sporangia of the younger ones swelled, turned gray, and then nearly black, and the vesicular bulbs of all increased in size. In no one of the five was there any sign of bending toward the light, although they were watched for 1 hr. and 40 min.

On the evening of May 21, 1910, observations were made on the reactions of three sporangiophores. The light was turned on at 7:38. The sporangiophores were inclined away from the direction of the light at an angle of 125°. The sporangial swelling in all of them was yellow. The vesicular bulbs had not begun to form. At 8:02 the sporangiophores had grown 1 mm. in length, but there was no change of position due to the presence of the light. The observation was continued until 9:20 P.M. The sporangiophores had not reacted toward the light, although they had continued their normal development.

At 9:20 P.M., January 19, 1911, observations were begun on six sporangiophores. They were in the same field. The light was turned on at 7:20 P.M. and observations were made for 2 hrs. One of the sporangiophores stood vertically. It was slender tipped and showed no signs of sporangial swelling. At 7:50, after 30 min. exposure to the light, the tips showed a very slight curvature. The reaction then stopped and the tip began to swell slightly. When the light was turned off, the sporangial swelling was distinct.

The remaining five sporangiophores at 7:20 stood at an angle of 135° to the incident light rays. All showed sporangial swelling, but the vesicular bulb had not started to form. There were no indications of response toward the light in any of them, although the sporangiophores continued their normal development throughout the experiment. At the close of the experiment the swelling of the vesicular bulb on all of them was just visible. Fig. 4 shows a sketch of these sporangia as they appeared at the beginning of the experiment.

At 7:27, January 20, 1911, the light was turned on a culture which had been exposed to the afternoon light, and observations were begun on five sporangiophores which were visible in one field. The first sporangiophore made an angle of about 80° with the direction of the light. It was slender tipped. At 7:58 the tip showed a slight increase in length and this portion was very slightly curved toward the light. At 8:23 there was a slight increase in curvature toward the light. At 8:42 the tip had curved through 35°, making an angle of 45° with the light. At 8:50 the curvature had increased so that the angle between the direction of light and the tip was only 25°. At 9:30 the tip was pointed directly toward the light. From this time the tip grew directly toward the light (fig. 5).

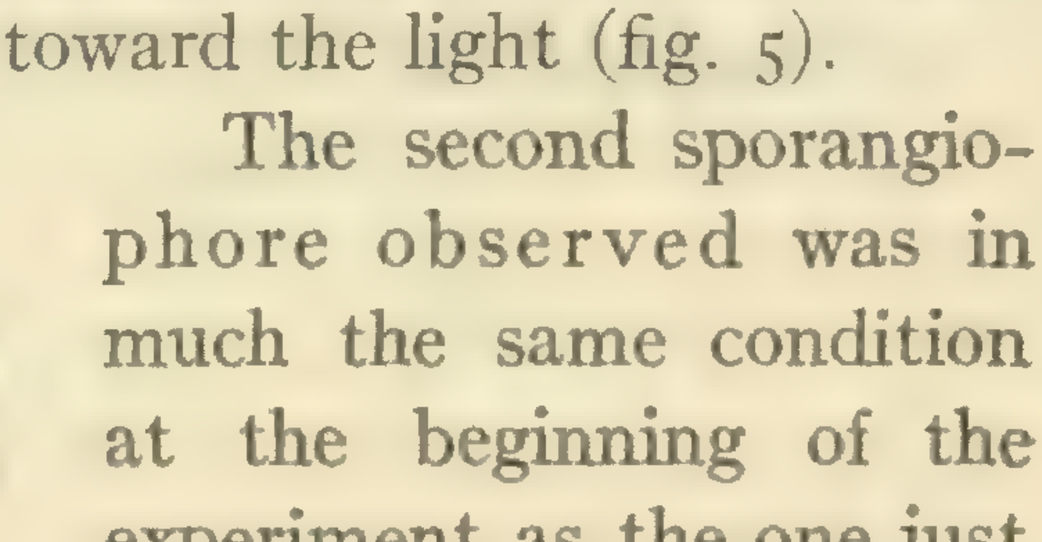

FIG. 4

The second sporangiophore observed was in much the same condition at the beginning of the experiment as the one just described, except that it was about 1 mm. longer. The reaction in this case was first noticeable at 8:00 P.M., the curve being barely visible. The curvature then proceeded more rapidly, and at 8:35 appeared more strongly than in the others in the field. At this time it had passed through an angle of 30°. At 8:42 the angle traversed was 45°. At 8:50 the angle made with the direction of the light rays was barely more than 10°, and at 9:13 the tip was directed straight toward the light and it continued in that direction until 9:50, when the observations were concluded. The portion beyond the curve was then about three-fourths of the length below (fig. 6).

FIG. 5

FIG. 6

The third sporangiophore was inclined at an angle of 80° from the incident light rays and measured 4 mm. in length. It was slender tipped. Curvature was first visible at 7:58 and at 8:42 was still very slight. At 8:50 there had been a slight increase in length, although no further change in direction was noted. At 9:40 the curvature became more pronounced and at 9:50 the tip was pointing almost directly toward the light. At this time the curvature seemed to be arrested. No further observations were made on this sporangiophore (fig. 7).

FIG. 7

The fourth sporangiophore observed at the same time stood at an angle of 80° with the direction of the light. It was 2 mm. in length and the tip was slender and tapering. The reaction in this case was strikingly like that of the third sporangiophore just described. They were very near together (fig. 8).

The fifth sporangiophore measured 1 mm. in length at the beginning of the experiment. It was slender tipped and stood vertically from the surface of the culture. At 8:04 the tip had begun to curve. At 8:42 the tip had curved through an angle of 40°. At 9:13 it pointed in the direction of the light (fig. 9).

FIG. 8

Observations were begun on two sporangiophores on one culture at 7:40, January 21, 1911. They were slender tipped and made an angle of about 50° with the direction of the light. At 8:18 both showed new growth which was curved slightly toward the light. The curvature continued with the growth until at 8:50 the tip was directed straight toward the light. From that time until 9:50 when the observations were concluded, the sporangiophores grew in the direction of the light (fig. 10).

FIG. 9

A group of five sporangiophores was located in the field of the horizontal microscope at 8:40 P.M., November 2, 1911. The sporangiophores showed only slight differences in length and were inclined at an angle of 25° from the vertical. They were placed so that they leaned away from the light, making an angle of 95°. The sporangial swelling was just visible on the tips of all the spo-

rangiophores. They were observed continuously, but there was no sign of a bending toward the light until 3:00 A.M. Meanwhile, the sporangial swelling had increased in size and the vesicular bulb was barely visible. At this time the sporangiophores were curved slightly at some distance below the sporangium nearer the direction of the lamp. Observations were not made again until 4:30, when the sporangia were aimed directly toward the light and the vesicular bulb was distinct; the curvature was in the region immediately beneath the vesicular bulb. At 6:30, when the observations were concluded, the bulbs had swollen considerably. The sketches (fig. 11) show the development of one of the sporangiophores of the group. The development of all the others were remarkably parallel with the one described.

FIG. 10

Observations were begun using a second horizontal microscope on a sporangiophore at 9:00 P.M., November 2, 1911. The tip of the sporangiophore was just beginning to swell. The sporangium was standing vertical to the surface of the substratum. The light was placed at the angle above mentioned. The development of the sporangial swelling continued, but no reaction toward the light was visible at 11:35. Observations were made continually until 3:00 A.M., when the sporangium was rather well formed and the vesicular bulb barely visible.

FIG. 11

On the evening of November 3, 1911, two sporangia were observed for the first time at 10:40. The first sporangiophore was yellow and rather blunt tipped, but as yet it did not show sporangial swelling. It stood at an angle of about 20° from the vertical, thus being inclined away from the light at an angle of 90°. There was no sign of a response toward the light at 12:00 P.M., but the tip then showed the sporangial swelling. At 2:05 A.M. the sporangiophore began to curve toward the light, the region of curvature being then located at some distance below the sporangial swelling. At 2:15 the curvature was more pronounced but still confined to the

same region. The bending continued until 5:30 A.M., when the tip of the young sporangium pointed directly toward the light. The entire portion between the region of bending and the young sporangium was swollen slightly, showing the beginning of the formation of the vesicular bulb. At 6:30 the vesicular bulb was large and turgid (fig. 12).

At the beginning of the observation the second sporangiophore in the same field of the microscope was somewhat longer than the first and the sporangial swelling was well formed. This sporangiophore also formed an angle of 90° with the light. The vesicular bulb was not yet visible. At 2:15 the sporangiophores had curved through an angle of 20° nearer to the direction of the incident light rays. The curvature in this case was also at some distance below the sporangium swelling, and the space between the two was

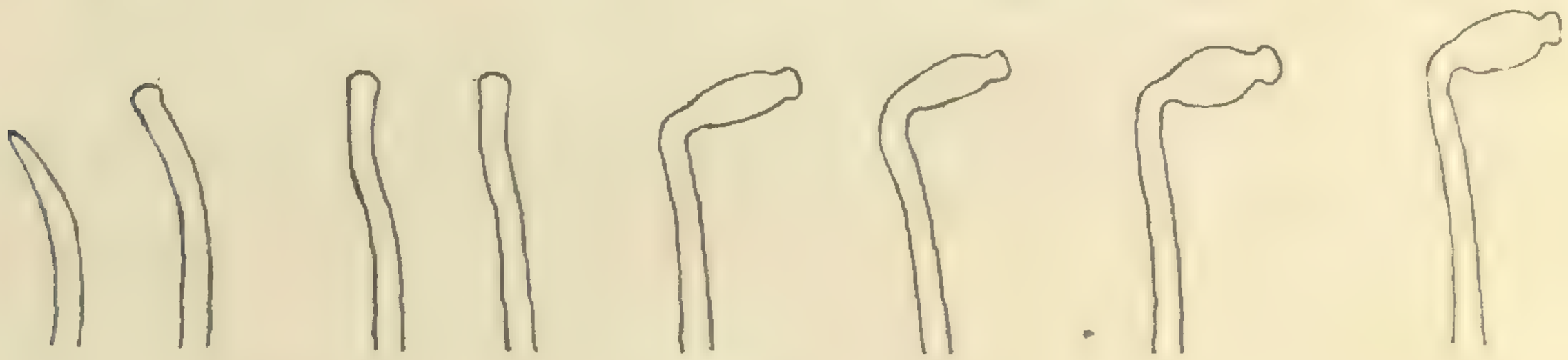

FIG. 12

beginning to show signs of the formation of the vesicular bulb. At 5:30 A.M. the tip of the sporangium was aimed toward the light. The vesicular bulb was still very inconspicuous. The bend in the sporangiophore was well rounded and had grown in length since the beginning of the curving. At 6:30 the vesicular bulb had swollen so that it exceeded the diameter of the sporangium by twice the diameter of the latter. At 8:00 the development appeared fairly complete. The sporangium was discharged between 9:50 and 10:00 A.M. (fig. 12).

From the foregoing experiments the following conclusions are evident:

1. Growth takes place at the tip in the young sporangiophore.
2. It is in the growing tip that heliotropic curvatures are formed.
3. In no case has a heliotropic curvature been observed during stages in early sporangium-formation.

4. Sporangium-formation may start during a reaction; in such cases the reaction is delayed for some time.

5. In case the reaction toward light is interrupted by sporangium-formation, it is resumed again a little before or about the time when the vesicular bulb is beginning to form.

6. After the sporangium is formed, the growing and curving portion is located immediately beneath the vesicular bulb.

The reaction of Pilobolus when exposed simultaneously to two equal sources of light

The effect of exposing a culture of *Pilobolus* simultaneously to two equal sources of white light was studied. For this purpose a light-proof box, measuring 120 cm. long by 60 cm. wide by 60 cm. high, was used. The box was made of pine and was painted a dull black on the inside. Running horizontally across one end, 20 cm. from the bottom, was an opening 10 cm. wide. Into the opening, which was prepared with rabbeted edges, was introduced a galvanized iron strip containing two openings 1 cm. in diameter and 9 cm. apart. The culture was placed at a distance of 25 cm. from the central point between the openings and on a level with them. It was placed with its surface vertical and facing the side of the box containing the openings.

The object of the experiment demanded that the light from the two openings be equal, but I know no methods of obtaining absolutely equal light intensities. We can only know that they are approximately equal. In order to obtain as nearly as possible equal illumination at the two openings, one light of measured intensity was placed equidistant from the two openings of the box and 40 cm. in front of it. Two mirrors were so adjusted that the culture intercepted the single spot of light formed by the convergence of the two sets of light rays. The light from either mirror was excluded from the opposite opening by means of the following device. At right angles to the edge of the box, along a line equidistant from the two openings, was placed an upright piece of board measuring 60 cm. high by 30 cm. wide by 1 cm. thick. At right angles to the first piece and parallel to the end of the box was nailed a second piece measuring 60 cm. high by 10 cm. wide by 5 mm. thick.

Both of the pieces were painted a dull black. The surface of the culture was covered with black paper, exposing a circular area 2 cm. in diameter. This small area was selected in order to exclude objectionable features such as unevenness in surface of culture, irregularity of distribution, etc. The number of sporangia was thus somewhat limited, but the undesirable features above mentioned were minimized.

This set of experiments was carried on in a dark room at the University of Wisconsin during April, May, and June 1910, under the direction of Dr. R. A. HARPER.

As previously described, a new set of sporangia matures daily and is discharged in the forenoon or early afternoon. The records of the results of the experiments were made daily in the late afternoon or evening. A glass plate fitting inside the box and against the openings caught the sporangia as they were discharged toward one or the other of the two lights.

The data were then recorded by means of a chart devised to meet the requirements of the experiment. The chart consisted of a large white sheet of paper divided by means of parallel lines into vertical strips 1 cm. wide. This is the principle of the Wolfhügel counter used by bacteriologists, and it was well adapted to the work in hand. The pieces of glass covering each of the 1-cm. openings fitted into the 1-cm. strips. In recording the data, the sporangia falling above and below the opening in the 1-cm. strip are recorded with those striking the opening. This is entirely fair, since, owing to the object of the experiments, we are concerned only with lateral distribution. Furthermore, our earlier experiments showed clearly the conditions of vertical distribution. The data for these experiments are recorded in table I.

In the first experiment 86 sporangia were discharged on the glass, 29 striking the vertical area containing the opening to the left and 25 that to the right. In the second experiment the total number was 60; 5 of these were on the area of the left opening and 20 on the right. In the third experiment 59 sporangia were counted on the glass, 5 and 18 being found on the left and right openings respectively. In the seventh all of the 22 sporangia discharged were fired toward the right opening, 10 of them striking the vertical

area containing the opening. Experiment 26 shows a total of 142 sporangia, 54 striking the vertical strip of the left opening, 30 that of the right opening. Experiment 27 shows 39 and 58 out of a total of 204 sporangia on the left and right openings respectively; and in experiment 28, 334 sporangia were discharged, 84 on the left and 78 on the right opening.

Throughout the series of experiments some of the sporangia failed to hit either opening. In experiment 1, 32 of the 86 sporangia discharged were of this sort. The distribution on either side of the two openings showed considerable variation. There were 9 sporangia in the 1-cm. strip to the left of the left opening and one sporangium in each of the next two strips. In the first 1-cm. strip to the right of the opening were 6 sporangia. On the left side of the right opening there were 11 sporangia in the first strip and 1 in the third; 3 sporangia were found in the first strip to the right of this opening.

In the second (table I), 3 sporangia struck the glass within 1 cm. to the left of the left opening. In the first, second, and third strips to the right of the opening were respectively 4, 2, and 1 sporangia. To the left of the right opening were 5 sporangia, all within the first strip. The number of sporangia in the first three consecutive strips to the right of the opening were respectively 18, 1, 1. The distribution and number of scattered sporangia in the remainder of the experiments showed about the same degree of variation as indicated by the complete data (table I).

In the foregoing experiments the number of sporangia which strike the openings does not appear especially large. These alone do not by any means give a complete conception of the accuracy of the total discharge of sporangia. A further knowledge of the distribution of these sporangia serves to correct the erroneous impression given by stating only the numbers that reach or do not reach the opening. The accuracy is very striking, for further consideration shows that 29 of the 32 sporangia that missed the opening in the first experiment were found within the 1-cm. strips on either side of the openings; only 3.5 per cent fell outside that area.

A perusal of the remaining data shows practically the same accuracy of discharge. The greater portion of the sporangia that

failed to strike the openings were found in the strips adjacent to the openings and only a very small percentage fell outside this area.

It is plain from these data that although there is much variation in the number of sporangia fired toward the two openings, the sporangia cluster about each of the openings and show no tendency to strike between the two. The sporangia are discharged, there-

TABLE I

Total									Opening									Opening						No. of experiments
86	...	...	...	...	...	1	1	9	29	6	...	...	...	...	1	...	11	25	3	...	...	...	...	1
60	...	...	...	...	...	...	...	3	5	4	2	1	...	...	...	...	5	20	18	1	1	...	...	2
59	...	...	...	...	...	...	1	5	5	3	...	...	...	...	...	3	10	18	10	2	1	1	...	3
56	...	...	...	...	...	...	...	1	2	1	...	...	...	2	2	5	14	14	13	2	...	...	...	4
25	...	...	...	...	...	...	...	3	8	1	2	...	1	...	1	...	1	7	...	...	...	1	...	5
41	...	...	...	...	...	...	...	2	9	4	2	...	...	2	1	1	4	10	4	...	1	1	...	6
22	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	2	4	10	4	...	1	...	...	7
35	...	...	...	...	...	...	...	4	7	1	1	1	...	1	...	4	5	7	3	...	1	...	...	8
45	...	...	...	...	1	1	...	5	13	3	3	...	...	1	...	1	1	11	5	1	...	...	...	9
36	...	...	...	...	...	...	...	5	7	2	...	...	...	1	1	2	1	12	3	...	1	1	...	10
54	...	...	...	...	...	...	...	6	3	3	...	1	...	...	2	1	6	21	7	3	1	...	...	11
29	...	...	...	...	1	...	1	5	3	1	2	...	...	...	...	...	3	13	...	...	...	...	...	12
24	...	...	...	...	...	...	...	2	4	2	1	...	1	1	...	...	...	11	2	...	...	...	...	13
24	...	...	...	...	...	...	...	1	9	3	...	...	...	...	...	...	1	8	...	1	...	...	...	14
14	...	...	...	...	...	...	...	1	4	...	...	...	...	...	...	1	3	4	1	...	...	...	...	15
24	...	...	...	...	...	...	1	3	7	2	...	...	...	...	1	1	3	6	...	1	...	...	...	16
20	...	...	...	...	...	...	...	2	3	3	...	...	...	...	1	2	2	3	...	3	...	...	...	17
12	...	...	...	...	...	...	...	...	4	...	...	2	...	...	1	...	1	2	2	...	...	...	...	18
20	...	...	...	...	...	...	...	1	6	3	...	1	1	...	...	1	5	1	1	...	...	...	...	19
7	...	...	...	...	...	...	...	...	2	2	...	...	...	...	1	...	...	2	...	...	...	...	...	20
35	...	...	...	...	...	...	1	3	10	5	2	...	...	...	...	3	4	7	...	...	...	...	...	21
27	...	...	...	...	...	...	...	2	8	2	4	...	...	...	...	2	3	5	1	...	...	...	...	22
14	...	...	...	...	...	...	...	...	5	1	1	...	...	...	...	...	...	4	3	...	...	...	...	23
22	...	...	...	...	...	...	...	1	4	3	...	...	2	...	...	...	...	7	5	...	...	...	...	24
34	...	...	...	...	...	...	...	6	12	5	...	...	...	1	...	...	2	6	1	1	...	...	...	25
142	...	...	...	...	1	...	4	16	54	17	1	1	...	1	...	...	10	30	6	1	...	...	...	26
204	...	...	...	...	2	3	4	23	39	9	6	3	4	1	1	7	20	58	20	4	...	...	...	27
334	...	...	...	1	1	2	3	24	84	23	5	4	2	6	3	9	34	78	39	8	2	3	3	28
133	...	...	...	1	...	...	2	8	43	12	2	...	...	1	3	6	16	32	4	2	...	1	...	29
178	...	...	...	1	2	1	8	26	52	28	6	1	1	2	1	2	16	21	8	1	...	...	1	30
146	...	...	...	...	1	...	4	12	25	8	4	2	2	...	3	4	27	44	7	...	1	...	1	31
24	...	...	...	...	...	...	1	2	2	1	...	1	1	1	...	...	4	9	1	1	...	...	...	32
252	...	1	2	3	2	3	5	25	25	16	8	5	4	5	5	12	22	44	33	9	9	8	6	33
61	...	...	...	...	...	2	2	5	2	5	3	4	2	1	1	4	7	13	4	3	2	1	...	34
30	...	...	...	1	2	1	1	3	2	2	...	...	1	3	4	2	3	2	2	1	...	...	...	35
176	...	...	...	1	2	5	17	15	9	6	...	3	...	8	13	25	20	19	13	5	8	4	3	36

fore, toward one or the other of the two lights. In other words, as shown by the earlier set of experiments, there is no resultant reaction due to the presence of the two lights. If there were, the sporangia would be for the most part between the two openings. It is clear, therefore, that this simple organism, when subjected simultaneously to two equal light stimuli, will respond to one of the two stimuli, to the complete exclusion of the other.

The sporangia, however, are not discharged in equal numbers toward the two openings. In the extreme case all might go to one or the other of the openings. It happened that in only one case (experiment 7, table I) did this occur, and then the total number was 22, the number being small and affording less chance for variation than would a larger number. The entire data thus show a great diversity of results as regards the number fired toward either opening. It is significant that the element of chance enters strongly into these results.

The fact that the sporangia do fire toward one or the other opening and in any degree of variation suggests further that although a large number of sporangiophores arise from one mycelium, they are not gregarious as regards physiological response toward light stimuli, that is, they act as separate individuals toward a light stimulus.

A further set of experiments was arranged to determine whether the sporangia to the right of the culture are discharged toward the right opening and those to the left of the culture to the left opening.

With the apparatus arranged as before, a second series of experiments was made with a thin plate of glass placed with its edge vertical to the middle line of the exposed surface of the culture. This glass would then intercept the sporangia discharged from the left side of the culture to the right opening and vice versa.

In the first experiment 2 sporangia struck each of the two openings, the number of sporangia in the consecutive 1-cm. areas to the left of the left opening are 8, 6, and 1; 3 sporangia were found on the 1-cm. area to the right of the opening. The first, second, and third 1-cm. areas to the left of the right opening contained respectively 1, 2, and 1 sporangia. The glass placed vertically at right angles to the surface of the culture received 7 sporangia on the left side and 15 on the right. The distribution of those on the left was as follows: 4 in the 1st cm. toward the culture, 2 in the 4th cm., and 1 in the 7th. The distribution of the sporangia on the right-hand side of the glass shows 7, 5, and 3 in the first 3 cm.

These data, together with those of the second and third experiments made in this series, are found in table II. These experiments tend to show that some of the sporangia on the right side of

the culture fire toward the left opening and vice versa. Some of this may be due to reflection from the glass.

TABLE II

The culture midway between and facing the two openings; a glass plate before the openings; a second glass plate at right angles to the surface of the culture. The data from the plates before the openings are given in the first part of the table; those from the plate at right angles to the surface of the culture are referred to by the marks *, †, and ‡.

Total								Opening								Opening							No. of experiment	Date
26	...	...	...	...	1	6	8	2	3	...	...	...	...	1	2	1	2	...	...	...	...	...	...	1*	6/15/'10 7:30 P.M.
7	...	...	...	...	...	...	1	...	...	...	...	...	2	...	...	1	2	1	...	...	...	...	...	2†	6/16/'10 7:35 P.M.
38	...	...	...	...	...	...	1	4	7	9	...	1	...	1	...	2	7	1	4	1	...	...	...	3‡	6/17/'10 7:20 P.M.

* Sporangia discharged on glass at right angles to surface of culture	4	...	...	2	...	...	1	...	...
	7	5	3	...	...	...	...	...	...
† Sporangia discharged on glass at right angles to surface of culture	6	3	...	...	...	...	...	...	...
	2	1	2	1	1	...	...	...	1
‡ Sporangia discharged on glass at right angles to surface of culture	1	1	...	2	1	...	...	...	1

Surface of glass to left of culture
" " " " right " "
" " " " left " "
" " " " right " "
" " " " left " "
" " " " right " "

The behavior of Pilobolus when given two equal sources of light but with the angle between the two sets of incident light rays varied

The box used for this set of experiments was made of redwood and measured 120 cm. long by 45 cm. wide and 35 cm. high. A hinged cover was so rabbeted as to be light-tight and was supplied with ice box catches in order to fasten it down tightly. At one end of the box, 14 cm. from the base, was an opening 9 cm. wide all the way across from side to side and so arranged with galvanized iron rabbets as to carry strips of galvanized iron 10 cm. wide which just closed the opening. These strips contained the openings through which the light was admitted to the culture. They were made of galvanized iron so that they would be thin enough to avoid shadows being cast by the rim of the openings. The openings were 1 cm. in diameter and their centers were the following distances apart: 1 cm., 2 cm., 3 cm., etc. The inner surface of the slide containing the opening was flush with that of the end of the

box, so that the glass plate on which the spores were caught fitted closely against the slide containing the openings. The openings not desired could be closed by means of two additional slides fitted in the groove from either end and outside the slide containing the openings. The inside of the box and of the strips was dull black.

The culture in this series was kept throughout the experiment at a distance of 23 cm. from the central point between the two openings. The surface of the culture was vertical and faced the slide containing the two openings. The exposed surface of the culture was 2 cm. in diameter. The light entering each of the openings was made to fall upon the exposed surface of the culture. The intensities of the light entering the two openings were equal or as nearly so as they could be made by measurement. The following device was followed in order to make the lights as nearly equal as possible. A single carbon filament incandescent lamp was placed at a distance midway between the two openings. By means of two mirrors placed at equal distances and equal angles one on either side of the lamp, the light was reflected through the opening to the exposed surface of the culture. In order to exclude the light of either mirror from the opposite opening a partition was set up in front of the box midway between the two openings, exactly similar to that used in the set of experiments last described.

With the change in the distance between the two openings it was necessary to change slightly the angles of the mirrors from the light in order that the spot of light reflected from the mirrors through the openings would strike the exposed surface of the culture. The condition of the experiments thus necessitated a slight change of light intensity from experiment to experiment, but the intensities of the light passing through the two openings at any one time were equal.

This set of experiments was performed in a dark room in the botanical laboratory of Leland Stanford Junior University.

In the first experiment, when the distance between the centers of the two openings was 5 cm., a total number of 38 sporangia were fired on the glass, 10 and 13 striking the left and right openings respectively. Only 2 of the 15 sporangia which failed to strike the opening were outside of the adjacent 1-cm. strips.

In the second experiment, with the center of the openings 4 cm. apart, 19 sporangia struck the glass; 10 were on the left opening, 7 on the right. Of the remaining 2 sporangia, 1 struck in each of the 1-cm. strips to the left and right of the left opening.

The third experiment shows a total discharge of 63 sporangia. Of that number 16 struck the left opening and 20 the right opening; 27 failed to reach either opening, but all of that number, excepting 3, were within 1 cm. of the opening.

When the distance between the openings was 14 cm., 50 sporangia were discharged, 15 striking each of the openings; 6 sporangia were counted within the first 1-cm. strip to the left of the left opening, and 1 in the third strip; 3 and 2 were within the first and second strip to the right of that opening; 5 sporangia struck within the 1-cm. strip to the left of the right opening, and 2 and 1 in the first and second strips on the right-hand side.

With a distance of 27 cm. between the two openings, 200 sporangia were discharged on the glass, 61 and 37 striking the respective openings to the left and right. The majority of scattered sporangia are again grouped around the two openings. The data for these experiments are tabulated in complete form in table III.

The results of the experiments in which the distances between the openings were varied agree with those obtained in the preceding set of experiments, where the openings were kept at the same distance throughout the set of experiments. The sporangia were fired with great accuracy toward one or the other of the two openings. The distribution of the sporangia about the openings varied in about the same degree as in the case just mentioned; and in the same way, the sporangia which are outside the vertical strips containing the openings are mostly within 1 cm. of one of the openings.

The reaction of Pilobolus when stimulated simultaneously by lights of different wave-length

The problems connected with the simultaneous stimulation of an organism by light rays of various wave-lengths offer an interesting field to the investigator. But I know of no way of accurately comparing lights of different colors as to the total amounts of

TABLE III

Distance between center of openings equals number of spaces between heavy figures plus one space.

| Total | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Number of experiment |
|---|---|---

radiant energy. The following experiments, therefore, are qualitative only; but I hope they will prove suggestive.

The different colors of the spectrum are represented in different proportions in the various incandescent lamps. These filaments are of standard make and the energy of the bulbs is measured in candle-powers. Thus by using bulbs of equal candle-power and current of known intensity we shall have somewhat comparable quantities.

Although this method is not all that could be desired, it has a very marked advantage over the colored solutions used by SACHS, and also the monochromatic glass plates that are so generally used in work on the effects of rays of various wave-lengths. The colored solutions absorb a large portion of the total energy emanating from the source, different solutions differing in this respect. In the case of the solutions (and it is also true of the colored plates) neither the intensity nor the energy of the lights can be compared. The incandescent lamps offer at least the advantage that they are of nominal commercial value; and with the advent of the knowledge of methods of comparison of intensities of colored lights, some exact idea of the intensities in the different parts of their spectrum may be obtained. Some study has been made to determine the distribution of the different wave-lengths in the different incandescent lamps, but so far it is insufficient for the present instance. It is known, however, that of the three incandescent lamps used in the experiments, the tungsten has the largest proportion of the actinic rays, the tantalum next, and the carbon least. The results with these filaments may thus serve to check up with those of the earlier work, in which the solutions and the plates of colored glass were used.

Experiments were made in order to test the relative efficiencies of different incandescent lamps in bringing about the reaction of *Pilobolus*. In these experiments the carbon filament, the tantalum, and the tungsten were compared. The experiments were performed in the dark room, using the redwood light-proof box already described. The openings were 1 cm. in diameter and the distance between them from center to center was 10 cm. The culture was placed 23 cm. from the point midway between the two openings.

The entire surface of the culture, which was 5 cm. in diameter, was exposed. Before each of the openings, at equal distances, was placed one of the two lights to be tested. The angle at which the lamps were placed was such that the spot of light from each lamp struck symmetrically the open surface of the culture.

The series of experiments was begun with a 32-candle-power carbon filament lamp before one opening and a 20-watt tungsten before the other. The first experiment gave a total of 92 sporangia; 31 sporangia were discharged toward the carbon filament and 61 toward the tungsten. In the second experiment 163 sporangia were discharged; 68 toward the carbon filament and 95 toward the tungsten. In the third experiment 387 out of a total of 784 sporangia went to the tungsten. Of the 1116 fired in the fourth experiment, 113 went to the carbon, 1003 to the tungsten. The next experiment shows a total discharge of 228 sporangia; 78 toward the carbon, 150 toward the tungsten. A new carbon lamp was put in the place of the old in the seventh experiment, but again a very much larger percentage was aimed toward the tungsten; 589 sporangia were fired, the ratio standing 168 toward the carbon as against 431 toward the tungsten. The data for this set of experiments are given in complete form in table IV. From this table a comparison of the accuracy of aim of *Pilobolus* toward the carbon and tungsten lamps can be made.

TABLE IV

Total	32-c.-p. carbon filament								20-watt tungsten													
92	...	...	...	...	...	...	6	19	6	...	...	...	...	...	...	3	7	42	9	...	...	...	...	...
163	...	...	...	...	...	1	3	47	14	2	1	...	...	1	1	3	15	54	20	...	...	1	...	...
784	...	1	1	...	...	6	64	203	86	15	6	4	2	2	5	3	40	253	88	3	...	1	1	...
1116	...	...	...	...	...	3	19	69	19	3	...	2	2	3	5	17	170	607	179	18	...	...	...	...
228	...	...	...	...	...	...	15	47	12	4	...	...	...	...	4	6	22	101	17	...	...	...	...	...
894	...	...	...	...	1	...	39	111	21	7	1	1	...	2	2	9	58	503	136	4	1	...	...	...
589	...	...	...	...	1	7	48	78	24	3	4	2	3	6	8	8	69	258	63	4	1	2	...	...

Of the 31 sporangia discharged toward the carbon lamp in the first experiment, 19 (61 per cent) struck the glass over the 1-cm. vertical strip containing the opening. Of the 61 sporangia

discharged toward the tungsten lamp, 49 (80 per cent) struck within the corresponding strip.

In the second experiment, of the 163 sporangia discharged, 68 were discharged toward the carbon filament, 47 (69 per cent) striking the 1-cm. strip over the opening; 95 were discharged toward the tungsten, 54 (56 per cent) on the strip over the opening. In this experiment, unlike the first, the larger percentage struck the strip over the opening in the case of the carbon filament.

In the third experiment, 387 sporangia were discharged toward the carbon, 203 (51.7 per cent) of them in the vertical strip containing the opening; 397 were discharged toward the tungsten, 253 (65.2 per cent) on the region of the opening.

The fourth experiment shows 69 sporangia, which is 61 per cent of the 113 sporangia discharged, toward the carbon, on the 1-cm. strip containing the opening; and 607 (61 per cent) of the 1003 sporangia fired toward the tungsten on the same region.

The remaining experiments of this series all show greater accuracy in the tungsten light than in the carbon filament light. With one exception, that of the second experiment, the discharge of the sporangia is more accurate toward the 20-watt tungsten used than toward the 32-candle-power carbon filament lamp, although the energy of the tungsten lamp is only half that of the carbon lamp.

Again, the percentages which strike the openings probably do not at first glance appear remarkable, but on noting in the first experiment that the 12 sporangia that did not strike the opening in the case of those fired toward the carbon light were all within 1 cm. of it, the accuracy is striking. Of the 19 sporangia which failed to strike the opening before the tungsten lamp, 16 were within 1 cm. of the opening and the remaining 3 were within 2 cm. of the opening.

In the second experiment, 21 of the sporangia fired toward the carbon light missed the vertical strip containing the opening; 17 of that number were within 1 cm. distance of the strip. Of the 41 sporangia that missed the opening in the case of those fired toward the tungsten, 35 struck within 1 cm. of the opening.

In the remaining experiments, most of the sporangia which failed to reach the opening in the strips fell within the first 1-cm.

strip on either side of them (table IV), showing the remarkable precision with which the sporangium is thrown toward a light.

A series of experiments was made to test the relative efficiencies of a tungsten and a tantalum lamp in bringing about a reaction of *Pilobolus;* 40-watt lamps of each kind were used. The tungsten was placed before the left opening, the tantalum before the right opening at a distance of 40 cm.

TABLE V

Total								Tungsten 40-watt										Tantalum 40-watt						
373	...	1	...	1	...	11	50	**137**	56	25	10	1	3	3	2	6	13	**31**	17	5	1	...	...	...
628	...	...	...	...	14	15	100	**243**	100	32	5	4	5	4	5	10	34	**34**	17	3	3	...	...	...
206	...	...	...	...	...	...	24	**71**	13	4	1	3	4	5	3	4	18	**39**	15	2	...	...	...	...
321	...	...	...	...	...	7	36	**104**	42	5	...	1	...	...	2	3	40	**61**	16	2	...	...	...	...
185	...	...	...	...	...	9	25	**61**	23	6	5	...	1	2	1	4	15	**24**	7	2	...	...	...	...
166	...	...	...	2	1	8	26	**67**	25	5	2	1	...	...	...	1	9	**13**	3	1	...	2	...	...
688	...	...	...	...	3	20	112	**291**	102	19	6	2	2	2	2	5	29	**47**	24	...	...	...	...	...
886	...	...	...	...	...	50	176	**363**	158	51	20	11	4	2	3	1	14	**22**	9	1	1	...	...	...
574	...	...	...	3	5	15	69	**248**	53	18	2	1	...	...	1	5	24	**43**	23	2	1	1	...	...
1091	...	...	...	6	20	46	156	**354**	164	46	21	8	7	8	13	20	40	**141**	51	15	5	...	...	...
464	...	1	1	5	2	21	81	**166**	71	29	17	3	2	3	3	8	7	**27**	9	4	2	2	...	...
367	...	...	...	...	1	17	82	**143**	28	25	15	7	1	...	1	4	8	**27**	7	1	...	...	...	...

In the first experiment, 373 sporangia were discharged on the glass, 294 toward the tungsten and 79 toward the tantalum. The second experiment showed a total number of 628 sporangia, with 516 and 112 toward the tungsten and tantalum respectively. The number of sporangia discharged in the third experiment was 206; of these, 118 were fired toward the tungsten and 88 toward the tantalum. The total number of sporangia in the fourth experiment was 321, 197 and 124 being fired toward the tungsten and tantalum. In the fifth experiment, 185 were discharged, 130 toward the tungsten and 55 toward the tantalum. The sixth experiment also showed a larger number had been fired toward the tungsten. The data for this set of experiments are found in table V.

The accuracy of aim toward the tungsten and the tantalum lamps was compared as in the preceding experiments. In the first experiment 46.6 per cent were discharged toward the tungsten, 39.2 per cent toward the tantalum. In the second experiment the

percentages fired toward the tungsten and tantalum were respectively 47 and 33.5. The third experiment shows 60.1 per cent before the tungsten and 44.3 before the tantalum; the fourth, 52.7 and 49.1 respectively; the fifth, 46.9 and 43.6; and the sixth, 48.9 and 44.8. The tenth and twelfth experiments show slightly larger percentages on the opening before the tantalum. The percentages figured out for the numbers striking either opening are given in table VI. The larger percentages strike the opening before the tungsten in these experiments. Most of the sporangia missing the opening strips in these experiments were found in the adjacent 1-cm. strips, as was the case in the foregoing experiments.

TABLE VI

Number of experiment	Percentages before tungsten lamp	Percentages before tantalum lamp	Number of experiment	Percentages before tungsten lamp	Percentages before tantalum lamp
1	46.6	39.2	7	44.3	42.7
2	47	33.5	8	41.3	40
3	60.1	44.3	9	59.5	43
4	52.7	49.1	10	42.9	47.6
5	46.9	43.6	11	41.7	40.9
6	48.9	44.8	12	44.2	56.7

In the next series of experiments a 40-watt tantalum was placed before one opening and a 20-watt tungsten before the other. The total number of sporangia fired in the first experiment was 1004; 731 were on the half of the glass toward the tantalum and 273 are on the half toward the tungsten.

The second experiment shows a total discharge of 407 sporangia, 329 on the tantalum as against 78 on the tungsten. The third and fourth experiments both show larger numbers on the tantalum. The data for this set of experiments are found in table VII. The accuracy of aim toward the two lights may also be obtained from the data in this table. Of the 731 sporangia discharged toward the tantalum lamp in the first experiment, 420 (54 per cent) struck the 1-cm. strip containing the opening. Of the 273 fired toward the tungsten, 149 (54 per cent) were on the corresponding strip. In the second experiment, the number of sporangia fired toward the tantalum lamp was 329; of these 161 (49 per cent) were in line with the opening. The total toward the tungsten was 78, with 37

(47 per cent) on the opening strip. In the third experiment, the ratio of the percentages of those on the 1-cm. strip containing the opening, in the case of the tantalum lamp, to that on the corresponding strip in the case of the tungsten is as 57 to 61. In the fourth experiment the accuracy of aim is very nearly the same toward the two lamps, although a greater proportion of the total number favor the tantalum lamp. The comparison of a 40-watt tantalum lamp against a 20-watt tungsten shows that a larger number of sporangia are discharged toward the tantalum, but that there is very little difference as regards the accuracy of aim toward the two lamps.

As in the set of experiments last described, a close examination of the data (table VII) reveals the fact that most of the sporangia that missed the vertical strips containing the openings were found within 1 cm. of them. An average of 7.8 per cent struck the glass more than 1 cm. laterally from the opening.

TABLE VII

Total								40-watt tantalum										20-watt tungsten						
1004	...	...	...	...	4	9	137	420	110	31	11	5	10	5	3	12	40	149	55	3	...	...	...	...
407	...	...	...	...	...	3	89	161	49	12	7	5	5	5	3	3	15	37	13	...	...	...	...	...
125	...	...	...	...	...	...	18	40	10	2	...	...	...	...	...	...	11	34	10	...	...	...	...	...
292	...	...	...	1	...	4	35	111	34	10	3	...	1	1	4	5	23	45	15	...	...	...	...	...

A 40-watt tantalum lamp was next compared with a 32-candle-power carbon filament with the results shown in table VIII. The first experiment gave a total of 96 sporangia, 36 of which were discharged toward the tantalum and 60 toward the carbon. The second experiment gave a total of 231 sporangia, with 164 fired toward the tantalum and 67 toward the carbon. In the third experiment 365 sporangia struck the glass, 268 over the tantalum and 96 over the carbon. In the fourth experiment, of the 157 sporangia fired, 98 were on the glass over the tantalum and 59 over the carbon. The fifth experiment showed a total discharge of 1039, 674 being found on the glass before the tantalum, 465 before the carbon. In the remainder of the experiments, as can be seen from

the data (table VIII), there is a larger percentage of sporangia over the tantalum. Thus, with the exception of the first experiment, the number discharged in the direction of the tantalum lamp surpassed that discharged toward the carbon.

TABLE VIII

Total								Tantalum 40-watt									Carbon 32 candle-power						
96	....	...	...	...	...	...	8	**23**	5	...	...	...	...	...	...	1	10	**40**	9	...	...	...	...	....
231	....	...	...	...	...	...	43	**79**	34	6	1	1	...	...	3	2	17	**32**	13	...	...	...	...	....
365	....	...	...	...	...	...	65	**136**	49	15	3	1	...	...	...	1	28	**48**	14	5	...	...	...	....
157	....	...	...	...	...	...	24	**57**	14	2	1	...	...	...	...	1	6	**40**	10	1	...	1	...	....
1039	....	...	...	...	...	7	116	**323**	107	15	3	2	2	2	1	15	91	**242**	195	8	...	...	...	....
76	....	...	...	...	1	2	12	**25**	1	1	3	...	...	...	...	2	6	**17**	6	...	...	...	...	....
404	....	...	...	...	...	1	35	**164**	29	1	...	...	...	2	1	1	26	**110**	31	3	...	...	...	....
1775	1	1	1	2	3	36	164	**748**	170	31	9	6	1	5	11	26	106	**283**	135	20	10	5	...	1
100	....	...	...	...	...	...	7	**47**	11	1	...	...	...	...	...	1	6	**23**	4	...	...	...	...	....
507	....	...	...	...	1	5	56	**154**	59	5	2	1	2	1	1	5	51	**121**	40	3	...	...	...	....
1442	....	...	...	1	3	8	208	**553**	246	24	8	2	2	7	6	28	85	**183**	63	6	2	3	...	2
813	5/11	4	4	10	15	20	58	**178**	77	15	15	17	16	18	25	31	65	**93**	71	18	18	9	5	11/4
174	....	...	...	...	1	3	29	**44**	8	2	1	1	1	3	2	7	15	**30**	22	7	...	...	...	....
249	....	...	...	1	...	3	20	**95**	18	1	1	...	...	...	1	3	30	**58**	17	1	...	...	...	....
111	....	...	...	...	...	...	11	**69**	17	1	...	...	...	...	...	...	2	**9**	2	...	...	...	...	....
104	....	...	...	...	...	...	10	**61**	17	1	...	...	...	...	...	...	3	**10**	2	...	...	...	...	....
184	....	...	...	...	...	...	19	**101**	19	1	...	...	...	...	...	...	7	**28**	9	...	...	...	...	....
194	....	...	...	...	...	...	22	**62**	27	1	...	...	...	...	...	...	6	**55**	20	1	...	...	...	....
123	....	...	...	...	...	...	12	**35**	8	...	...	...	...	...	...	2	10	**42**	14	...	...	1	1	....
1231	....	...	...	2	14	40	118	**266**	162	40	18	2	2	1	2	50	122	**257**	107	42	9	3	...	1
44	....	...	...	...	...	...	5	**19**	4	1	...	1	...	...	...	1	1	**11**	1	...	...	...	...	....
43	....	...	...	...	...	1	5	**16**	...	...	...	...	...	...	1	...	2	**12**	6	...	...	...	...	....
561	....	...	...	...	...	6	98	**146**	35	5	5	5	2	1	2	8	56	**122**	44	7	...	...	...	....

Of the 36 sporangia discharged in the direction of the tantalum lamp in the first experiment, 23 (63.8 per cent) struck the glass within the 1-cm. strip containing the opening. Of the 60 sporangia fired toward the carbon, 40 (66.6 per cent) struck within the 1-cm. strip containing the opening. In the second experiment, 79 (48.2 per cent) of the 164 sporangia that are discharged toward the tantalum are found in the 1-cm. strip over the opening; 32 (48 per cent) of the 67 discharged toward the carbon are on the corresponding strip. In the third experiment the percentages which strike the glass on the 1-cm. vertical strip over the opening before the tantalum and carbon filament lamps are respectively 50.7 and 50. In the fourth experiment 58 per cent of the sporangia fired toward the tantalum and 67.7 per cent of those fired toward the carbon filament lamp strike the strip containing the respective openings.

The above percentages striking the area of the 1-cm. opening before each of the two lamps compared are given, together with those computed for the remaining experiments, in table IX. The complete data from which the percentages were figured are found in table VIII.

TABLE IX

PERCENTAGES OF THE TOTAL NUMBER OF SPORANGIA DISCHARGED TOWARD THE TANTALUM AND CARBON FILAMENT LAMPS WHICH STRUCK THE 1-CM. VERTICAL STRIPS COVERING THE OPENING IN EACH CASE IN EXPERIMENTS 1–23 IN TABLE VIII

Number of experiment	Percentage before tantalum lamp	Percentage before carbon lamp	Number of experiment	Percentage before tantalum lamp	Percentage before carbon lamp
1	63.8	66.6	13	49.3	34.8
2	48.2	48	14	68.3	52.7
3	50.7	50	15	60.2	69.2
4	58	67.7	16	68.5	66.7
5	68.1	52	17	78.5	63.1
6	55.5	58	18	55.3	67
7	71.2	63.2	19	63.6	61.8
8	63.8	47	20	32.6	43.1
9	71.2	67.6	21	63.3	78.5
10	57	54.2	22	72.7	52.3
11	52.4	47.6	23	45.6	50.4
12	41.7	24.2			
			Average..	58.6	55.9

In this set of experiments the accuracy of discharge varies considerably. In some cases the discharge toward the tantalum is more accurate; in others that toward the carbon lamp is more so. On the whole, however, the discharge is a little more accurate toward the tantalum, but the difference is so small that it is practically negligible.

From the percentages in table IX, it stands out clearly that wherever there was a small percentage which struck the opening before one lamp, there was usually a comparatively small percentage on the corresponding area before the second light. In the first experiment, 63.8 and 66.6 were the percentages striking the two openings. In the second experiment, 48.2 and 48 per cent struck the openings. In some cases there is less uniformity. The eighth experiment shows 63.8 per cent on one opening and 47 per cent on the other. But on the whole, a small percentage on

one opening is usually accompanied by a corresponding percentage on the other. It might be suggested that this may be due to the general condition of the culture at the time of the experiment. The accuracy might be affected by food supply, moisture, temperature, and other factors of importance to the physiological condition of the sporangiophores at the time of stimulation.

Here, as in the previous experiments, the sporangia that strike outside of the opening are to be found for the most part in the first vertical strips to the left and right of the openings. An examination of the data (table VIII) will show a good proportion of the cases where all that were fired toward a single light are found on the opening or adjacent strips. Thus it is clear that the accuracy is much greater than it would appear from an examination of table IX alone.

Summary

Physiologists, in studying the reactions of plants to stimuli, have for the most part worked with phototactic organisms or organisms of considerable complexity, individuals in which there was a differentiation of tissues, where the cells in one portion of the body may receive a stimulus, another perceive it, and still another respond to it. Such a study has the disadvantage of dealing with too many factors and accompanying phenomena. In *Pilobolus* the reaction is marked and can be easily studied. A single cell receives the stimulus and responds to it. The protoplasm of the cell receives the stimulus, perceives it, and reacts. The accuracy of response of *Pilobolus* toward the light is remarkable, when we consider its size and the distance through which it throws its sporangia. The sporangiophore scarcely ever exceeds 1 cm. in length, and is usually somewhat shorter, while the distance through which it discharges the sporangium in most of the experiments is over 25 times that measurement. The accuracy of response and the nicety of organization of such a mechanism can well be appreciated from the study of such experiments. From such work the capacities of a single cell can best be realized.

The results of the experiments in which *Pilobolus* is stimulated simultaneously by two lights bear directly on NOLL'S (4) theory

of "heterogene induction." According to NOLL, the reaction of an organism to one of two stimuli excludes the effect of the second stimulus. His work was concerned with two very different kinds of stimuli, light and gravity. The reaction to the stimulus of light excluded any response to the stimulus of gravity. Recent workers, GUTTENBERG (2) and RICHTER (5), have interested themselves along the same lines. GUTTENBERG maintains that if the light stimulus be diminished sufficiently, a resultant reaction between light and gravity will occur; that NOLL and the earlier workers had used light that was too intense. To RICHTER'S (5) criticism that his results were due to impure air, GUTTENBERG (3) responded by further work under improved conditions and reached essentially the same results as before.

In experiments on simultaneous stimulation of *Pilobolus* by lights of the same kind or of different kinds, we are dealing, unlike either of the foregoing cases, with simultaneous stimuli of one kind, namely, light alone. It was possible to have the stimuli at least approximately equal, and it was possible to have the arrangement such that neither source of stimulation had any advantage over the other. Further, the organism worked with was a simple one, the reaction concerning only a single cell. And the net result of the reaction was shown so plainly in the distribution of the discharged sporangia that it seems impossible that any indefiniteness or uncertainty could be entertained as to the reaction. The sporangia clustered always about one or the other of the two sources of illumination. There was no sign of a resultant reaction. Even if the individual sporangiophore did not receive equal illumination from the two openings, if there were any resultant reaction, it would be expected that the sporangium would be found in a position between the two lights, depending on the ratio of their intensities, differences of composition, and the like. Thus, it would be expected that all of the sporangiophores would be located between the two sources of illumination. Such a condition did not obtain in any case where the two light stimuli were used, whether or not they were the same as far as distribution in the spectrum was concerned. The sporangiophore reacted to one or the other of the two stimuli. The results obtained at least suggest that one stimulus does not

affect the reaction to the other. To this degree the results are corroborative of NOLL's theory. But NOLL's theory, based on his work with light and gravity as stimuli, suggests a change of geotonus due to the presence of the light. In working with the two light stimuli, the reaction to one of the two stimuli to the exclusion of the other cannot be explained in this way, although here, as above, the plant is subjected to two directive influences.

Where the two simultaneous stimuli were of different kinds, gravity and light, NOLL believed that light may call forth certain changes in the plasma which, directly or indirectly, cause the reaction. He says that perception and reaction may rest on entirely different characters; but the reaction may be carried out by the same changes. In work where the two simultaneous stimuli are of the same character, as in the present experiments, the reaction is less complicated. Thus but two sets of changes of the plasma need be concerned with the response.

The above experiments show that there is no sign of a resultant reaction, but that they can in no way determine whether, so far as perception is concerned, there is any influence of one light on the other. According to NOLL's theory, a change of geotonus takes place, due to stimulation by light; that is, there is a change of sensibility to gravity as such due to the perception of light. The question arises whether, with the presence of two light stimuli of the same kind acting through the same time, there is a corresponding change of sensibility toward one light owing to the mere presence of a second of a similar kind. Apparently there must be some factor or factors somewhere in the organization of the plasma that brings about a total neglect of one stimulus and a complete reaction toward another of a similar nature, since the reaction is not a resultant one. It appears that NOLL's theory alone is insufficient to explain entirely the lack of resultant reaction to two directive forces when applied to an organism as contrasted with the results obtained by physicists in working with inanimate matter. The question, then, of what determines the reaction toward one light and a lack of response toward the second is still unsettled, and the explanation must be deferred to a time when more is known of the intricate mechanism and ultimate organization of the plasma of the cell.

In the experiment with the different incandescent filament lamps, as with the solutions and plates of colored glass used in the earlier work (1), *Pilobolus* fires its sporangia in larger numbers toward the lights in which the proportion of the blue rays is greatest. In other words, it is more responsive to actinic rays. The intensities in the different wave-lengths, as earlier mentioned, are not measurable; but the uniformity of response in favor of the source containing the greater proportion of actinic rays suggests the superiority of the more refrangible rays over the less refrangible rays in causing heliotropic curvatures. This question can be definitely settled only when the intensities of lights of different colors can be measured.

The energy given off by the source of light apparently does not compare in effect with the distribution of the same in different portions of the spectrum. In the experiments using a 20-watt or 16-candle-power tungsten lamp and a 32-candle-power carbon filament lamp the large majority of the sporangia went to the tungsten, although its total energy was but half that of the carbon. From this it is apparent that differences in distribution in the spectrum outweigh in effect the differences in the total energy of the two sources.

The set of experiments using a 16-candle-power tungsten against a tantalum of twice the number of candle-powers showed the discharge to be in favor of the tantalum. At first glance it appears that this contradicts the above results with the carbon and tungsten lamps, and suggests that the total energy of the source does play an important rôle in the results. However, on further consideration, it must be noted that the total number of actinic rays in a tantalum lamp of twice the intensity of the tungsten is probably greater than that in the tungsten. The solution of this point, of course, is bound up with the question of intensity and composition of the sources under discussion and cannot be taken with any degree of finality. With the tungsten lamp of approximately the same intensity as that of the tantalum, the discharge was in favor of the tungsten which emits a larger proportion of the blue rays. The difference as far as distribution in the different wave-lengths of the energy of the tantalum and tungsten lamps is not so great

as is the case of that in the carbon and tungsten lamps. This may account for some of the differences in the distribution of the sporangia in the two cases, when comparing the tungsten with the carbon and with the tantalum.

A comparison of the carbon and tantalum lamps shows again a majority of sporangia discharged on the opening before the tantalum lamp which contained the larger proportion of blue rays.

In the experiments with the rays of different wave-lengths, although the stimuli are both light stimuli, there is a marked difference in composition. We have then an extra factor to deal with; but, as in the experiments with the two nearly equal light sources, there is no sign of a resultant reaction. There is no sign of a change of aim toward one light owing to the presence of a second light. With the tendency of the sporangiophores to discharge toward the blue light, however, it is plain that there is no uniform aim of all the sporangia subjected to the two lights to go to the light having the larger proportion of the blue rays. Why does not *Pilobolus* always discharge toward the source of light having more of the actinic rays? The difference in the length of the light ray does bring a marked variation as regards the numbers fired toward the two sources. Still, some are fired toward the less favorable of the two sources.

The accuracy of aim toward the two lights might well be said to agree in general with that already found for the two light sources used in the above experiments. However, there is a noticeable difference in accuracy of aim toward the different filaments, and that for the most part is in favor of the light with the larger proportion of the more refrangible rays. With the solutions and glass plates used in the earlier work (1) there was a much greater difference noted. There is a probability that the smaller difference may be due to less difference in light intensity, to a smaller difference in composition, and also that there is a limit to the accuracy of response toward any source of stimulation, and that in aiming at the lights in use in these experiments they reached that limit, the less effective lamp being sufficient, or in some cases nearly so, to bring about as accurate a reaction as is possible to the plant.

The question as to what properties of the protoplasm cause it to be more sensitive to rays of one wave-length than to those of another remains unsolved, and, like that as to why it responds to one of two stimuli to the complete exclusion of the other, it must await a better knowledge of the organization of the plasma.

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MORPHOLOGY OF THISMIA AMERICANA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 182

NORMA E. PFEIFFER

(WITH PLATES VII–XI)

The family Burmanniaceae, chiefly tropical in distribution, is represented by about 50 species in EUBURMANNIAE, 2 in CORSIAE, and 18 in THISMIAE. The geographical range of the first named group is by far the widest; its representatives are found in all tropical regions and extend into the temperate zone. In North America, they are found as far north as Florida, Alabama, and even Virginia. CORSIAE are reported for New Guinea and Chile. THISMIAE are represented by two monotypic genera, *Glaziocharis* and *Triscyphus*, recorded for Brazil, and by 15 species of *Thismia*, if zygomorphic forms are included.

Thismia is subdivided into four groups, *Euthismia*, *Geomitra*, *Bagnisia*, and *Afrothismia*. The *Geomitra* and *Bagnisia* divisions had been described as separate genera by earlier workers, but have recently been included in the genus *Thismia*. To date, the following species in this genus have been described in these regions:

Thismia Brunoniana Griffith (21), Tenasserim; T. Gardneriana J. Hooker (2), Ceylon; T. macahensis B. & H. (*Ophiomeris macahensis* Miers) (27), Rio de Janeiro; T. hyalina B. & H. (*Myostoma hyalina* Miers) (28), Organ Mts.; T. Aseroe (*T. Ophiuris*) Beccari (1), Borneo and Singapore; T. Neptunis Beccari (1), Sarawak; T. javanica J. J. Smith (9), Java; T. Winkleri Engler (7), Africa; T. crocea Ernst (*Bagnisia crocea* Becc.) (1), New Guinea; T. episcopalis F. Muell. (*Geomitra episcopalis* Becc.) (1), Borneo; T. clavigera F. Muell. (*Geomitra clavigera* Becc.) (1), Sarawak; T. clandestina Miq. (*Sarcosiphon clandestina* Blume) (12), Java; T. Rodwayi F. Muell. (29), Tasmania; T. Hillii (*Bagnisia Hillii* Cheesem.) (3), New Zealand; T. Versteegii J. J. Smith (12), Java.

Of these, the first 7 are of the *Euthismia* type, the eighth is the sole representative of *Afrothismia*, and the rest are of the *Bagnisia* or *Geomitra* group. The distribution of these is seen to be practically restricted to the Polynesian Malay region. In view of

this, the finding in the Chicago region of a form closely allied with these last is of decided interest.

Thismia (BAGNISIA) **americana,** nov. sp.—Herbae saprophyticae, tenerae, hyalinae, caulibus simplicibus erectis, radicibus elongatis glabris foliis bracteiformibus. Pedunculi uniflori, erecti vel curvati, 0.3–1 cm. longi. Flores subtiliter virides 0.8–1.5 cm. longi, circiter 6 mm. diametro. Perianthii tubus superus, obovoideo-oblongus, ore constrictus, lobis 6, quorum interiores tres apice conniventes, calyptram 3-stipitatam formantes; lobi alterni equales sed liberi. Stamina 6, fauci affixa, intra tubum deflexa filamentis brevissimis, connectivis maximis membranaceis in tubum deflexum connatis; antherae biloculares, loculis parvis distinctis parallelis, rima longitudinale dehiscentis. Ovarium breve, latum, 1-loculare, placentis 3 parietalibus, in cavo ovarii a pariete solutis. Stylus brevis, crassus, apice trifidus. Ovula numerosa, minuta, anatropa. Fructus turbinato-cupulatus, perianthii circumscisse deciduo truncatus, margine parum elevato cinctus. Semina numerosa, parva, oblonga, albuminosa; testa tenuis, hyalina, reticulata. Embryo parvissimus, in albumine inclusus.

Chicago, Ill., in open prairie, N. E. PFEIFFER.

The plant consists of a white root system, from which arise erect simple floral axes. The roots are about 1 mm. in diameter and vary greatly in length. The flowers are 0.8–1.5 cm. high, borne on an axis 0.3–1.0 cm. high. The perianth tube is conspicuously 6-nerved and with 6 minor nerves. The 3 petals, approximately equal in length to the 3 sepals, are connate at the apex. The mouth of the perianth tube is closed by a disk of tissue, with a central circular aperture surrounded by a raised ring. To this ring the 6 stamens are affixed, and are united into a tube which hangs downward inside the perianth tube. This stamen tube, largely made of the broadened connectives, bears the pollen sacs on the side toward the perianth wall.

The inferior ovary is one-celled, with three placentae which soon become free from the walls, appearing in a central plane as three free columns. The ovules are anatropous, numerous, and

small. There are two integuments. The seed contains a few-celled embryo imbedded in a mass of endosperm.

The entire plant is glabrous and white, save in the 6 divisions of the perianth, where free, and in the disk closing the perianth mouth. Here there is a delicate blue-green color, deeper in the raised ring about the aperture of the disk. Most of the plants have only this colored upper portion above the level of the soil, or of the surrounding moss, etc. The diameter of 5–6 mm. and a height above the soil of 4–6 mm. give an idea of the size of the flower. When the soil is carefully removed, the underground parts are found to be white and semi-transparent; they lie more or less parallel to the surface of the soil, at a depth of a few millimeters. There is no connection with other plants, although the roots of *Thismia* often lie in close juxtaposition with other roots. When the plant is so exposed (figs. 3 and 4), the flower plainly shows the typical THISMIAE structure; a tubular, 6-parted perianth, with the three inner members united at the apex. The leaves, as in other dependent Burmanniaceae, are reduced to white scalelike bracts, so closely appressed to the floral axis that they are readily overlooked.

The material was first discovered in August 1912, in a small space along the margin of a grass field. The habitat may be described as a low prairie, characterized by such plants as *Solidago serotina*, *S. tenuifolia*, *Rudbeckia hirta*, *Eupatorium perfoliatum*, *Asclepias incarnata*, *Iris versicolor*, *Acorus calamus*, and *Agrostis alba vulgata;* and on the soil itself *Selaginella apus*, *Aneura pinguis*, and *Hypnum*. Usually the *Thismia* grows in spots where the soil is not closely covered by *Aneura* and *Selaginella*, but it may be found occasionally among the moss (fig. 3). The little plant is evidently protected both against strong light and great transpiration; but its habitat is in striking contrast to that of most of the other species of *Thismia*, which are found in rich-loamed primeval forests, in regions of great rainfall.

The plants were watched for stages in development during August and the first half of September, to the time when some fruits were obtained. In the season of 1913, visits to the field were made weekly, with the result that flower buds were found on July 1, about a month earlier than the first observation of the previous season.

The indications were that the underground parts had wintered over, although seed-germination may have occurred and been overlooked, since the flower is all that appears above ground.

Earlier descriptions of the Burmanniaceae gave little attention to any but the gross features, which were in the main correctly interpreted. Until recently, the work in anatomy has been done largely by JOHOW (**25, 26**) in *Apteria setacea*, *Gymnosiphon refractus*, *G. trinitatis*, and *Dictyostegia orobanchioides*. The saprophytic forms worked with have scaly rhizomes, from which the flower stalks arise directly, as in *Thismia*. The adventitious roots are reported as being simple, with corky endodermis and a single, greatly reduced vascular bundle of lignified, dotted vessels, arranged in two concentric rings about one central spirally thickened element. The rhizome is described as having a structure much like that of the root. The erect stem or floral axis is credited with having bundles showing distinct xylem and phloem in most forms. The exceptions are *Apteria* and *Gymnosiphon trinitatis*, which have such small bundles that the differentiation is difficult to recognize, according to JOHOW. Nevertheless, he reports all cells of the bundle as lignified.

Recently ERNST and BERNARD (**9–20**) have added largely to the knowledge of the anatomy of different saprophytic forms. They have considered *Thismia javanica* J. J. Sm., *T. clandestina* Miq., *T. Versteegii* J. J. Sm., *Burmannia candida* Engl., *B. Championii*, Thw., and *B. coelestis* Don (*B. javanica* Bl.). In these forms, the vascular elements in the root are much reduced; in *T. javanica* (**10**) the bast alternates with thin-walled parenchyma cells about a central woody area, which is separated from the former by parenchyma cells. The xylem region is figured as consisting of as many as 13 vessels. The fungus occurs in these roots in a patchy arrangement, infecting one group of cortical cells and not another. In the uninfected cells starch is common.

In *T. clandestina* and *T. Versteegii* (**13**), a similar situation as regards arrangement of vascular elements is found, but the xylem is not so conspicuous in amount. In the former species, there is but one subepidermal layer of fungi, in the latter two, the outer of which is the coarser.

The xylem in *Burmannia candida* Engl. (**16**) is represented by but two spirally thickened cells in the central cylinder; occasionally there is only one, seldom three. In addition, there are parenchyma cells and phloem. *B. Championii* (**16**), in contrast to this, has a central cylinder largely made up of xylem elements, with no phloem evident. *B. coelestis* (**19**), a chlorophyll-containing form, shows a similar simple situation as to root anatomy.

In all the forms investigated, the floral axis has well developed collateral bundles. The vascular cylinder is sometimes surrounded by a ring of sclerenchyma tissue, as in *Burmannia candida* and *B. Championii*. The endodermis is usually distinct. Some of the cortical cells contain raphides. In *Burmannia candida* some of the surface cells are much like stomata in form, with pores always open. In *B. coelestis*, a chlorophyllous form, there are normal functioning stomata, in contrast to the usual lack in saprophytic forms.

In *Thismia americana*, superficial examination of the underground structures shows a relatively large number of buds in all stages of development. These occur not only on the main structure, which would appear to be a rhizome, but also on the structures which are very evidently roots appearing at the base of the floral axis. On examination of prepared sections, it appears that the histology of the main structure and of these secondary roots is identical, even to the appearance of a cap at the tip. Because of this fact, these structures, whether primary or secondary, will be referred to as roots. It would appear in field material that roots originally secondary might later appear primary by the dying away of a portion which thus severs the connection with the mother plant.

In the older part of a root of *Thismia*, there is evident a very conspicuous epidermis (figs. 7, 14). This consists of large cells, more or less protuberant, but not developed into hairs in any region. The epidermal cells, in contrast with the cortical cells below the surface, are hyaline. The layer of cells immediately below the epidermis is packed with the thick-walled, branching mycelium of a coarse fungus. In fresh material the mycelium

appears brown. The septate hyphae are usually oriented with the long axis of the root, so that the cross-section of a root shows numerous cut ends (fig. 14), and the longitudinal section a more or less parallel arrangement of the interweaving hyphae (fig. 7). Below this single layer is a region of a varying number of cortical cells containing much finer, thin-walled hyphae. In these deeper parts, masses of protoplasm are frequently evident which strongly suggest the sex organs of some of the Peronosporales. These undoubtedly correspond to the "vesicles" reported by JANSE (24) in *Thismia clandestina* (*T. javanica* J. J. Sm.), which he was inclined to believe asexually reproductive bodies. BRUCHMANN considered similar bodies in *Lycopodium annotinum* to be oospores of *Pythium*. In *Thismia* there are also bacteria, probably corresponding to JANSE'S "sporangioles" and "spherules." All these fungal parts are intracellular. A few cells outside of the endodermis are free from fungi. These contain raphides which are common throughout the plant body.

The endodermis consists of a single layer of heavy-walled cells, larger than their neighbors. It encircles a region, probably conducting, of which only a few central cells (3–5) are spirally thickened (figs. 5, 6, 19). They are not lignified, however, and the spiral markings are very fine. Near the point of origin of a floral axis, the number of thickened cells is increased and lignification occurs. These vessels may be seen to connect directly with the vascular elements of the floral axis. The cells adjacent to these spirally thickened vessels do not show the dotted condition that JOHOW reported in the Burmanniaceae considered by him. On the contrary, though they are somewhat elongated, they are nucleate and retain their cytoplasm. They are undoubtedly parenchyma cells, and so the situation is similar to that in *Thismia javanica* J. J. Sm. and other forms investigated by ERNST and BERNARD.

In the outer part of the conducting region are seen a varying number of points of small cells devoid of contents (fig. 6). The arrangement is similar to that reported in other forms by JOHOW and by ERNST and BERNARD. It suggests a radial arrangement, with these groups of cells probably reduced phloem strands without sieve plates.

The growing region resembles that in any root. In a few millimeters at the apex there is a meristematic region of actively dividing cells. Then there is the region of elongation and differentiation. The origin of the different layers would seem to conform to the general situation in monocotyledons, with distinct initials in calyptrogen, dermatogen, plerome, and periblem. This would be in contrast to the situation in *Thismia Versteegii*, where ERNST and BERNARD report a common initial for epidermis and root cap. In *Thismia americana* the tip region of the root is free from fungi, but the tissue formed is rapidly invaded by the mycelium from older parts. In no case was new mycelium seen to penetrate the epidermis and so enter the uninfected region, as reported by JANSE in *Thismia clandestina* (*T. javanica* J. J. Sm.).

Compared with that of the root, the anatomy of the floral axis is complex (figs. 8–10). Here the vascular elements are arranged in a cylinder of 3–6 bundles. In the early stages the number is very apt to be 3; in the older axis, near the apex, there are divisions of the original bundles, giving a larger number, frequently 6. More may be seen where branches go from the original bundles.

Each bundle consists of definite xylem elements and a mass of cells with slightly thickened walls (fig. 10). The xylem is slower to appear than the latter, which are early clearly distinguishable (fig. 8). In mature parts, the small clear cells appear in the same relation to xylem as phloem usually does, but they show no sieve plates so far as can be determined. Nevertheless, it seems probable that these elongated cells function as phloem. The xylem vessels have lignified and spirally thickened walls and their number varies from 2 to 15 in each bundle. The large number, the conspicuous spiral thickenings, and the lignification, as well as the greater and more definite phloem development, are quite in contrast to the condition found in the root.

A single strand of a few xylem vessels and phloem cells supplies each of the bractlike leaves (fig. 11), which are very thin and relatively broad. There are also branches of the main cylinder supplying the floral parts and producing the conspicuous nervation of the perianth.

There is no definite endodermis or pericycle in the floral axis,

nor is there a ring of sclerenchyma about the vascular cylinder as reported for some other burmanniaceous forms. As might be expected, no stomata were found; the corollary of no air spaces follows logically.

The floral axes appear to arise a short distance back of the tip of the root (figs. 4, 20). The first external evidence of their development is a single small excrescence (fig. 20*a*). Later two growing points, usually point upward, are distinguishable. Growth is quite rapid and soon results in a first root and a floral axis (fig. 20), from the base of which other roots take origin (figs. 21, 22).

In prepared material, the earliest stage in the development of the bud is shown in fig. 12. A region of rapidly dividing cells occurs below the epidermis in a somewhat arched mass. At this stage the main root itself is in such an undifferentiated condition that the endodermis and neighboring tissues are not yet distinct. In slightly older stages, a break is seen to occur between the cortical cells of the root and the growing cells of the endogenous branch, which now has a slightly lobed margin (fig. 13). At this time, the beginning of the first root to be developed may be seen (figs. 13, 19) as a mass of meristematic tissue to one side of the floral bud. By rapid growth the root overtakes the stem from which it originated, so that when the two structures have emerged a little beyond the boundary of the main root, they are about the same size (figs. 14, 15). At this time the floral part is still protected by the arch of primary root cortical tissue, but the root tip soon breaks through the cortex, becoming much the longer organ (fig. 16). A renewed growth of the floral axis and the development of other secondary roots from the main floral axis (fig. 17) finally result in a horizontal position of the first root, which had previously stood erect beside the floral axis.

In the development of the axis, the rudiments of the bract leaves appear laterally (fig. 17). After elongation and the development of the leaves, the differentiation of the floral parts occurs (fig. 18). The perianth tube develops early. At the same time, the stamens begin their development. The ovary with its ovules is late in appearance, a case similar to that in Orchidaceae. When

the ovules are first beginning to be evident, the microsporangial tissue is well defined.

There are, as usual, four microsporangia in early stages (fig. 23), which later fuse to form the two pollen sacs (fig. 28). The early stages show the stamens bent inward and downward, but not connected with each other. Later growth, particularly of the connectives, makes a continuous tube, which is, however, easily separated into the constituent stamens (fig. 28). This tube extends beyond the sporangia, which occur on the side toward the perianth wall. In mature stages the pollen grains are slightly oval. They are very pale, almost transparent, with a pale green cast, due to little bodies, probably fat, many of which also occur in the perianth parts. The microspores are loose and free, not massed together as in pollinia of orchids. Before shedding, the generative nucleus divides (fig. 29). A granular mass in the spore suggests the presence of a prothallial cell, but failure to secure stages in the present investigation must leave this doubtful at present.

The ovules, very many in number, develop on parietal placentae (fig. 24), which swing free from the ovary walls in the center (fig. 18). At this level ovules project on all sides of the placental column. In early stages the numerous primordia appear as in fig. 18. The fully developed ovule shows two integuments (figs. 26–27). It is anatropous, with a long funiculus. As JOHOW (**26**) and TREUB (**30**), and lately ERNST and BERNARD, reported, there is a conspicuous differentiation of a few of the nucellar cells at the base of the embryo sac. This seems to have no significance at present.

The seed is minute, with a testa two cells in thickness. The outer layer is composed of large, almost transparent cells. The inner one is constructed of smaller cells, with more contents, often appearing oil-like. The seed has a very evident endosperm, with cells of relatively large diameter, and an inconspicuous embryo of a few cells (fig. 30). In all respects it seems to agree with the accounts of TREUB (**30**) and JOHOW (**25, 26**), the former of whom first correctly interpreted the endosperm. MIERS, in 1866, declared that the seed of *Myostoma* contained no embryo. Later, GRIFFITH interpreted the entire content of the seed as embryo. TREUB,

in 1883, found a weakly developed embryo of 3 or 4 cells in a mass of endosperm in *Burmannia maburnia* and *B. javanica.* JOHOW, in 1885, reported a similar situation in *B. capitata* with a 10-celled embryo, and in *Apteria* one with 4 cells. In *Thismia javanica,* ERNST and BERNARD (**11**) have found a more strongly developed embryo with 4–6 tiers of cells. *Thismia clandestina* (**14**) has a still better differentiated embryo, with a 3-celled suspensor above a spherical body, the outer cells of which are differentiated from the inner. *T. Versteegii* (**14**), a closely related form, has on the other hand a simple embryo. *Thismia americana* then would seem, in its embryo situation, to resemble this last species and forms like *Burmannia javanica* and *B. maburnia.*

No case of polyembryony has been found, such as was reported by ERNST (**8, 20**) in *Burmannia coelestis* Don, a form developing embryos apogamously. Here the number of embryos was one, two, or three, dependent on whether the egg alone functioned, or the synergids were also active. In the earlier history ERNST found no reduction division to occur in the formation of "mega-spores." Stages have not yet been obtained in *Thismia americana* to work out the sequence here, but assuredly, at maturity, only one embryo in each seed has so far been found.

The arrangement of parts in the flower seems such that insect pollination would be necessary, unless a situation similar to that in *Burmannia candida* Engl. and *B. Championii* existed. Here the pollen grains germinate in the sporangia, and the pollen tubes grow toward the style branches. No indication of this condition was found in *Thismia americana.*

Up to date, the few attempts at germinating the tiny seeds have been fruitless. It is to be hoped that a larger harvest may give a better opportunity for positive results. The relation of the fungus inhabitants to the developing plant might be better worked in this connection than with the mature plant. Since the fungi occur in the root, the absorptive region, and not in the stem, they would seem to have some connection with water and food supply. Microchemical tests show that in the root there is a very large supply of reserve food in the form of oils or fats. Contrary to the results of ERNST and JOHOW, no sugars or starch are present in

large enough amounts to detect by microchemical means. As indicated above, large amounts of calcium oxalate are present in the form of raphides throughout the parenchyma tissues of the plant body. This is probably to be related to the presence of the fungi.

The green oil-like bodies in the perianth parts gave in spectroscopic tests an absorption in the blue band. The coloring matter is not easily soluble in alcohol.

Further features in the morphology and cytology of this plant will be presented in a later paper.

Summary

1. The characters of *Thismia americana* are deemed sufficiently different from those of other members of the genus to warrant the description of a new species.

2. The main subterranean structure cannot be distinguished from a root, having a similar anatomical structure, including a root cap.

3. The root shows great reduction. The xylem is represented by 3–5 central spiral elements, the phloem by 4–6 small groups of cells. A radial arrangement is suggested in the grouping.

4. The vascular cylinder of the floral axis consists of 3–6 bundles, with xylem and phloem collaterally arranged. The xylem is composed of spiral lignified vessels. No sieve plates are distinguishable in the phloem.

5. The floral axis and first root arise from the main root endogenously. Other secondary roots arise from the base of the floral axis bud.

6. The ovary is slower in development than the other floral parts. Microsporangia are well developed when ovules first appear.

7. The ovules are anatropous, and have two integuments.

8. The embryo, consisting of a few cells, is imbedded in a mass of large-celled endosperm.

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EXPLANATION OF PLATES VII–XI

All figures, except 1, 2, 3, 4, 20, 21, 22, and 28, were drawn at the level of the table with the aid of a Spencer camera lucida under Spencer objectives 16, 4, or 1.8 mm., and oculars 2, 4, or 8. The following abbreviations are used: *b*, bud; *br*. bract; *e*, epidermis; *fa*, floral axis; *ii*, inner integument; *oi*, outer integument; *lt*, leaf trace; *l*, fungus infected layers; *m*, microsporangia; *mr*, main root; *o*, ovary; *p*, phloem; *p′*, perianth; *pw*, perianth wall; *rc*, root cap; *s*, style; *sr*, secondary root; *s′r′*, second secondary root; *x*, xylem.

FIG. 1.—Side view of plant of *Thismia americana*, in situ; ×2.

FIG. 2.—View of flower from above; petals cut apart at apex and folded back; ×4.5.

FIG. 3.—View from above of group of flowers undisturbed in natural situation; the oldest flower appears at the right; ×4.5.

FIG. 4.—View from above of plants from which the soil has been removed; the white root portions are evident with their buds; ×2.6.

FIG. 5.—Longitudinal section of central cylinder of root; ×266.

FIG. 6.—Cross-section of central cylinder of root; ×266.

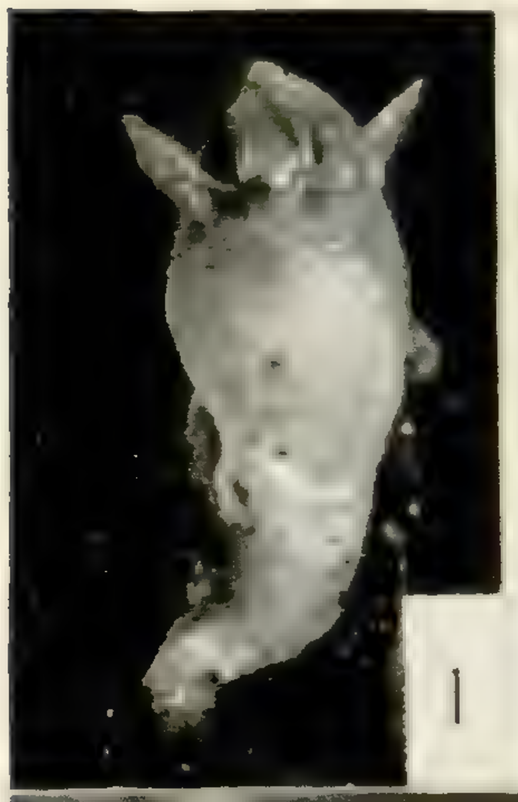
FIG. 7.—Portion of root in longitudinal section, showing subepidermal fungus infected layers, and epidermis free from fungi; ×266.

FIG. 8.—Cross-section of central region of young erect axis, showing early phloem development; ×266.

FIG. 9.—Diagrammatic cross-section of erect axis and bract leaves; the outlined portion of the axis proper is shown in fig. 10, of the leaf in fig. 11, with higher magnification; ×35.

FIG. 10.—Detailed drawing of portion of cross-section of floral axis; one leaf trace supplies the next leaf; ×266.

FIG. 11.—Detail of portion of cross-section of leaf, showing single, simple bundle; ×266.



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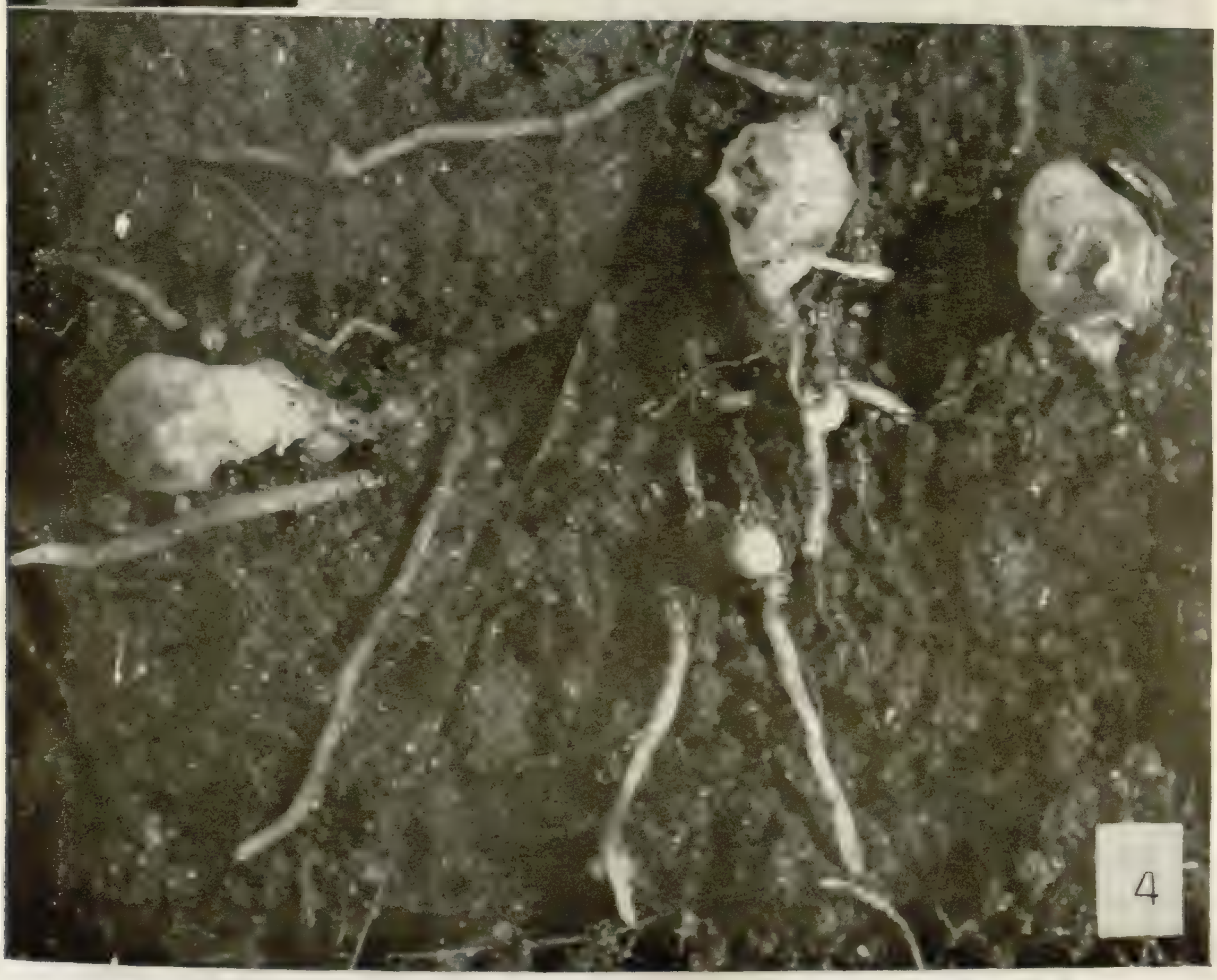

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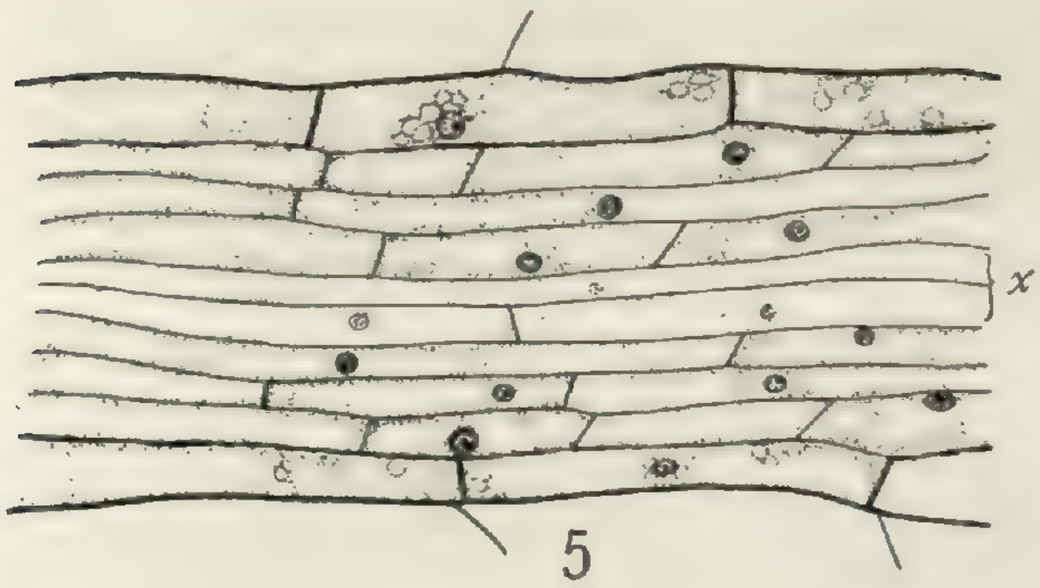

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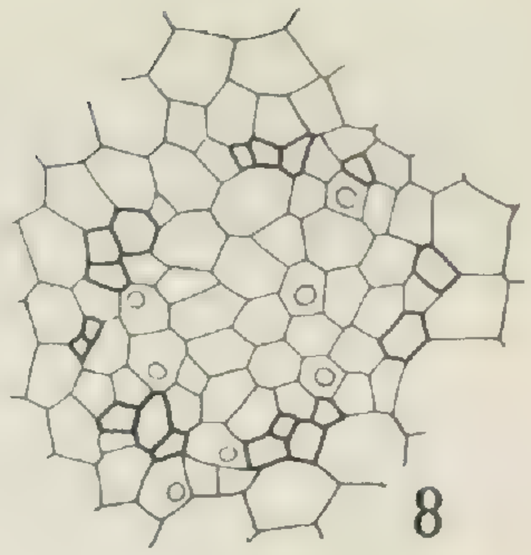

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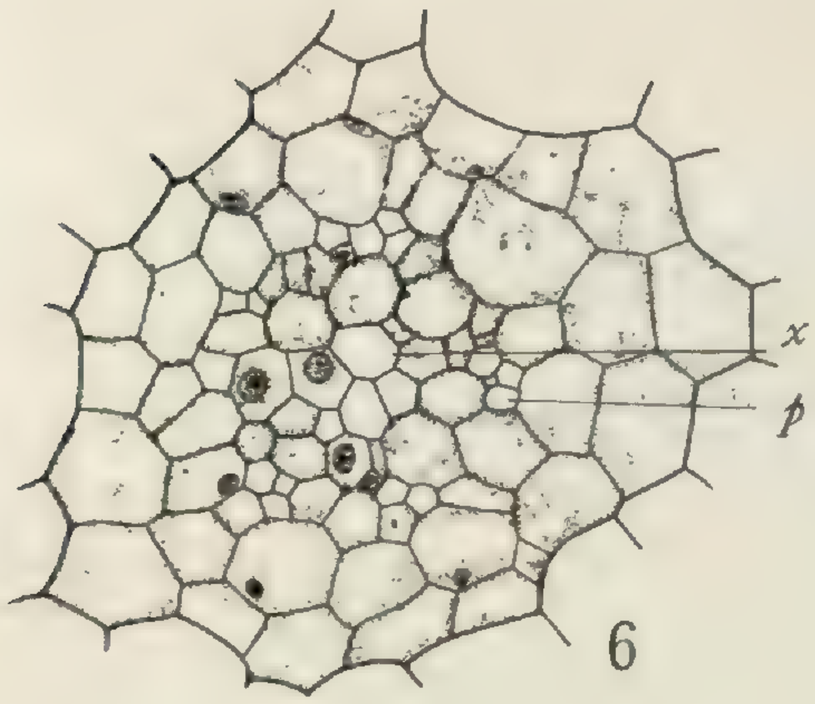

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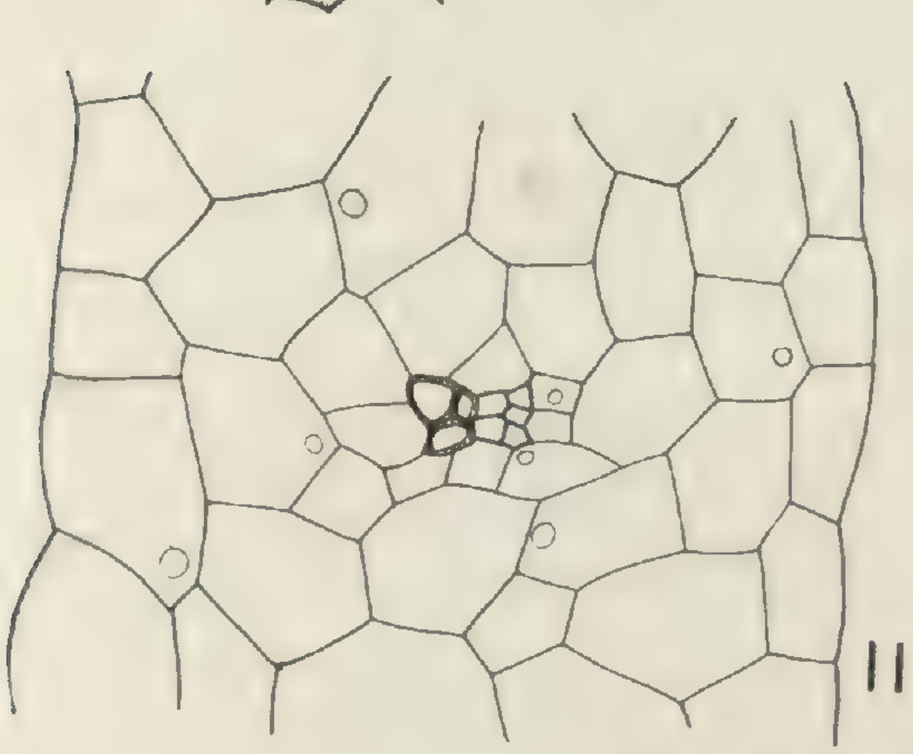

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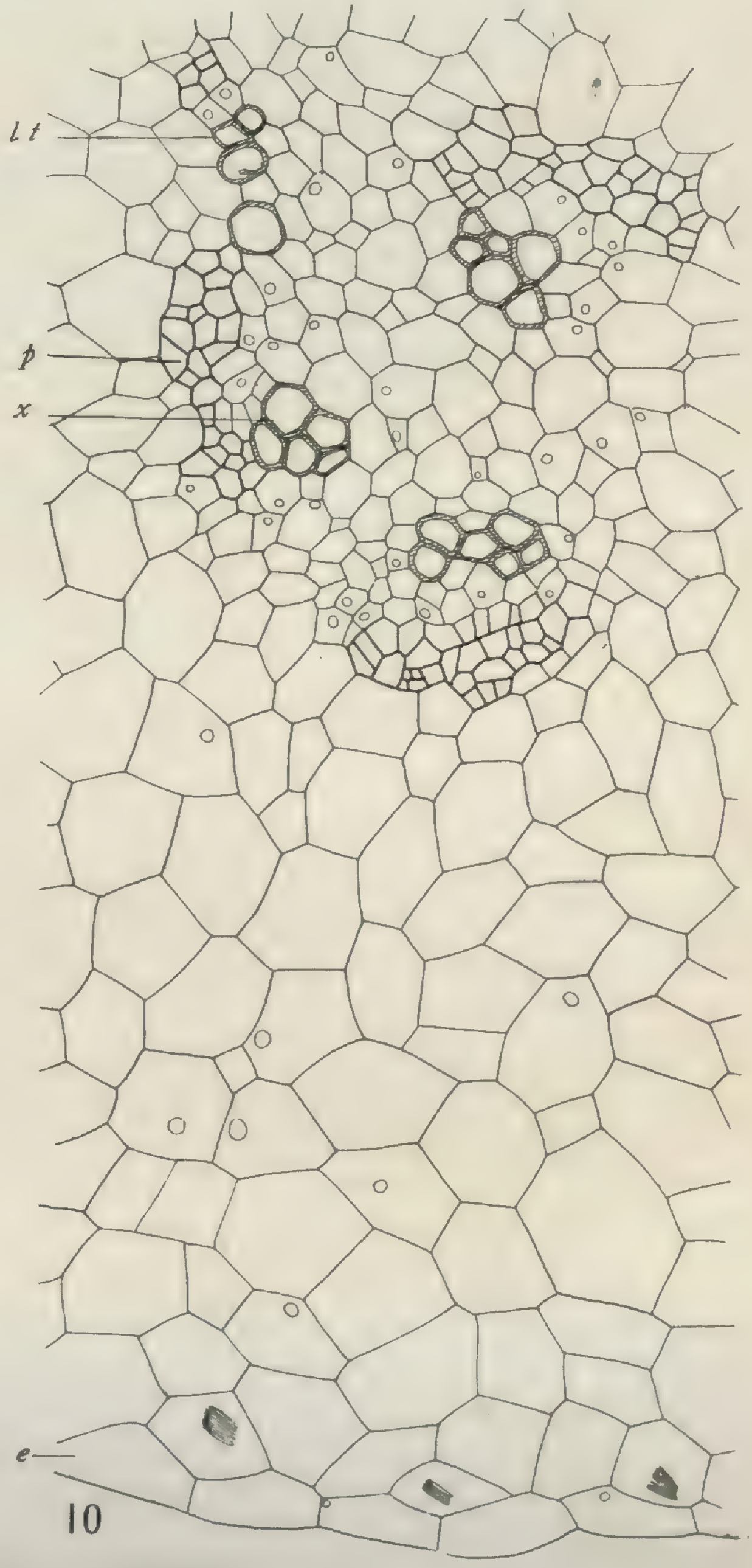

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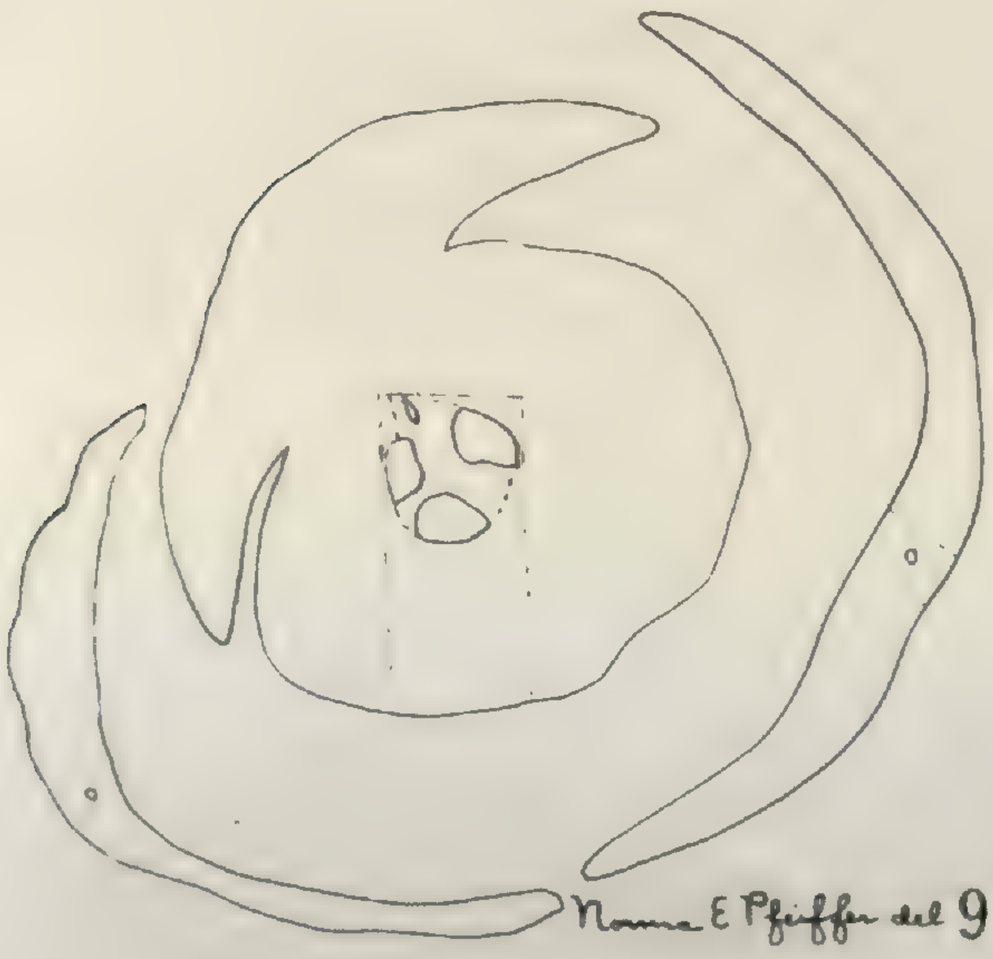

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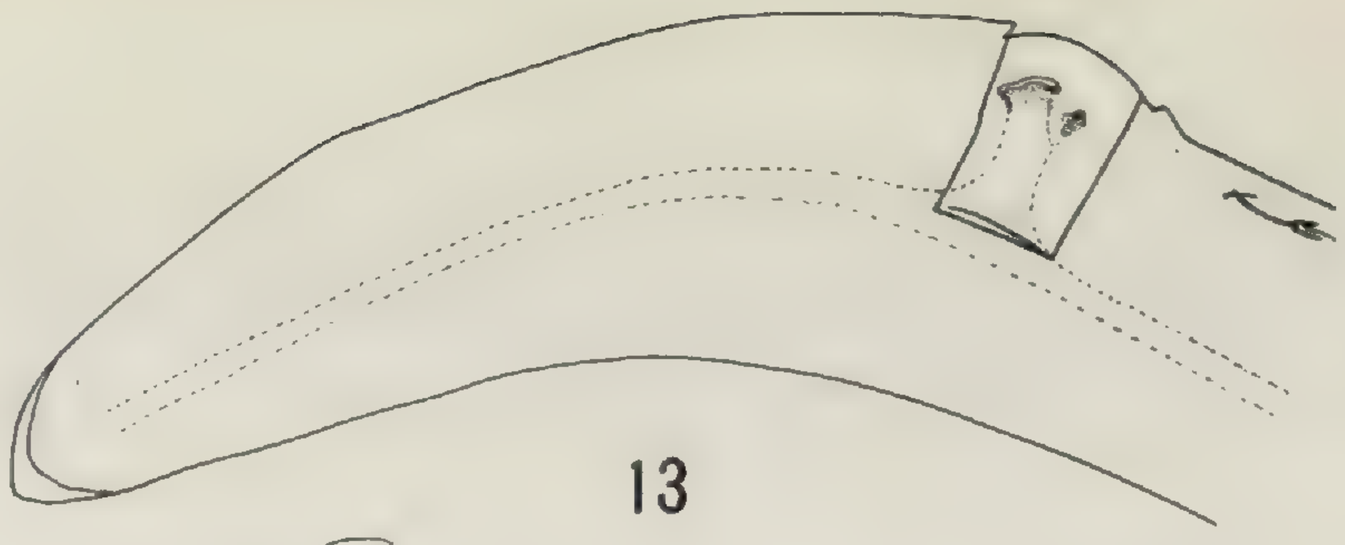

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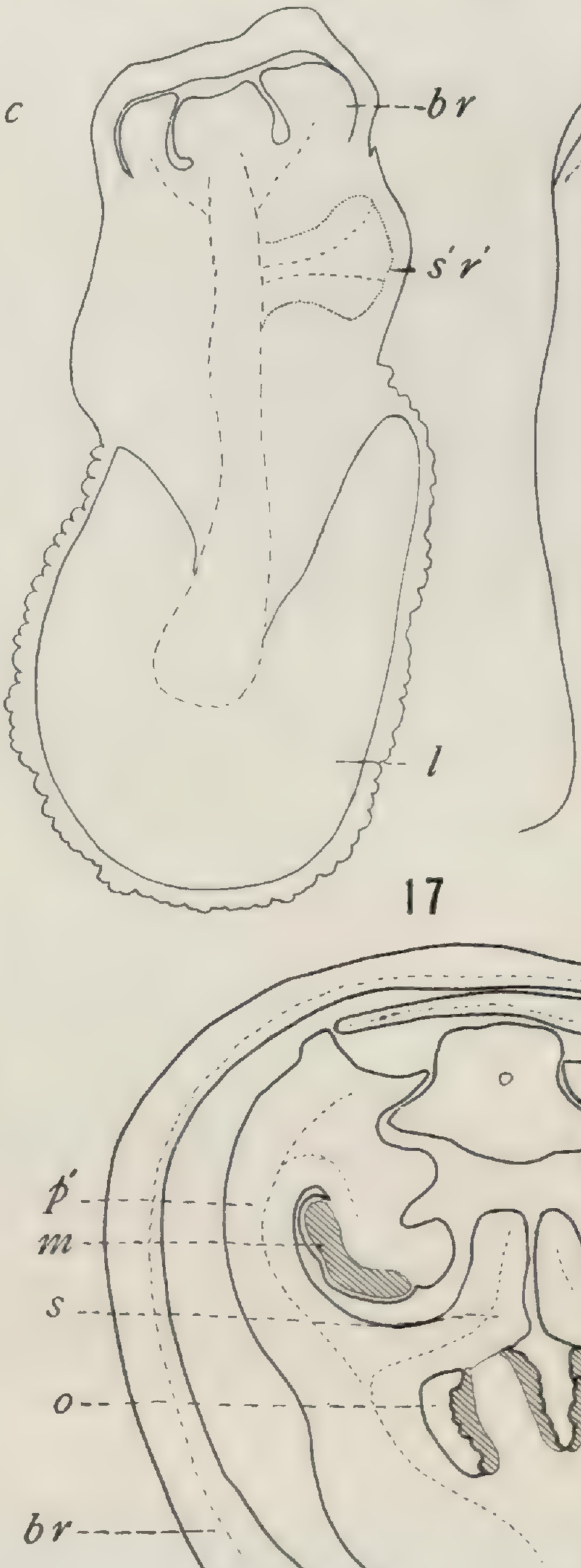

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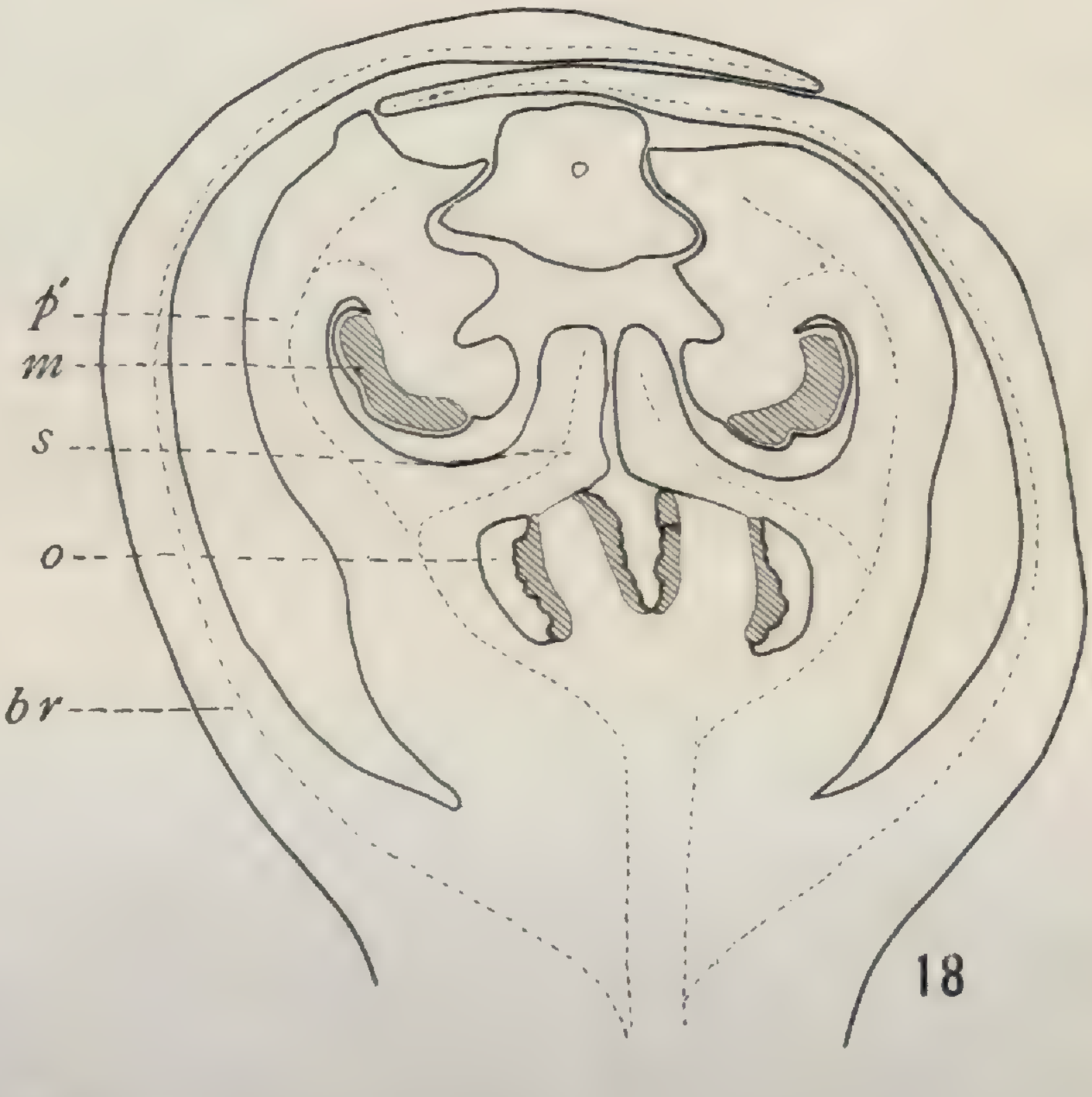

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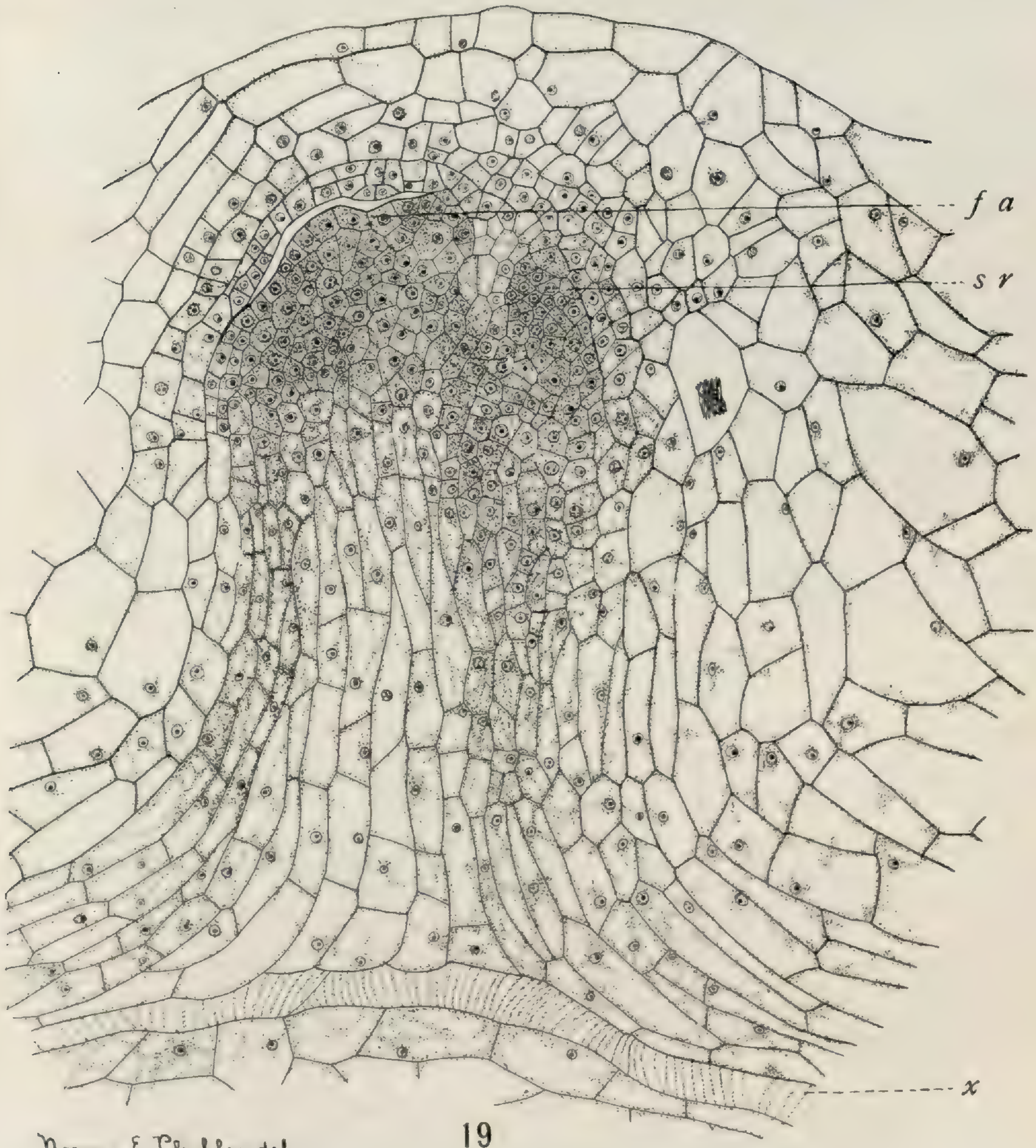
PLATE X



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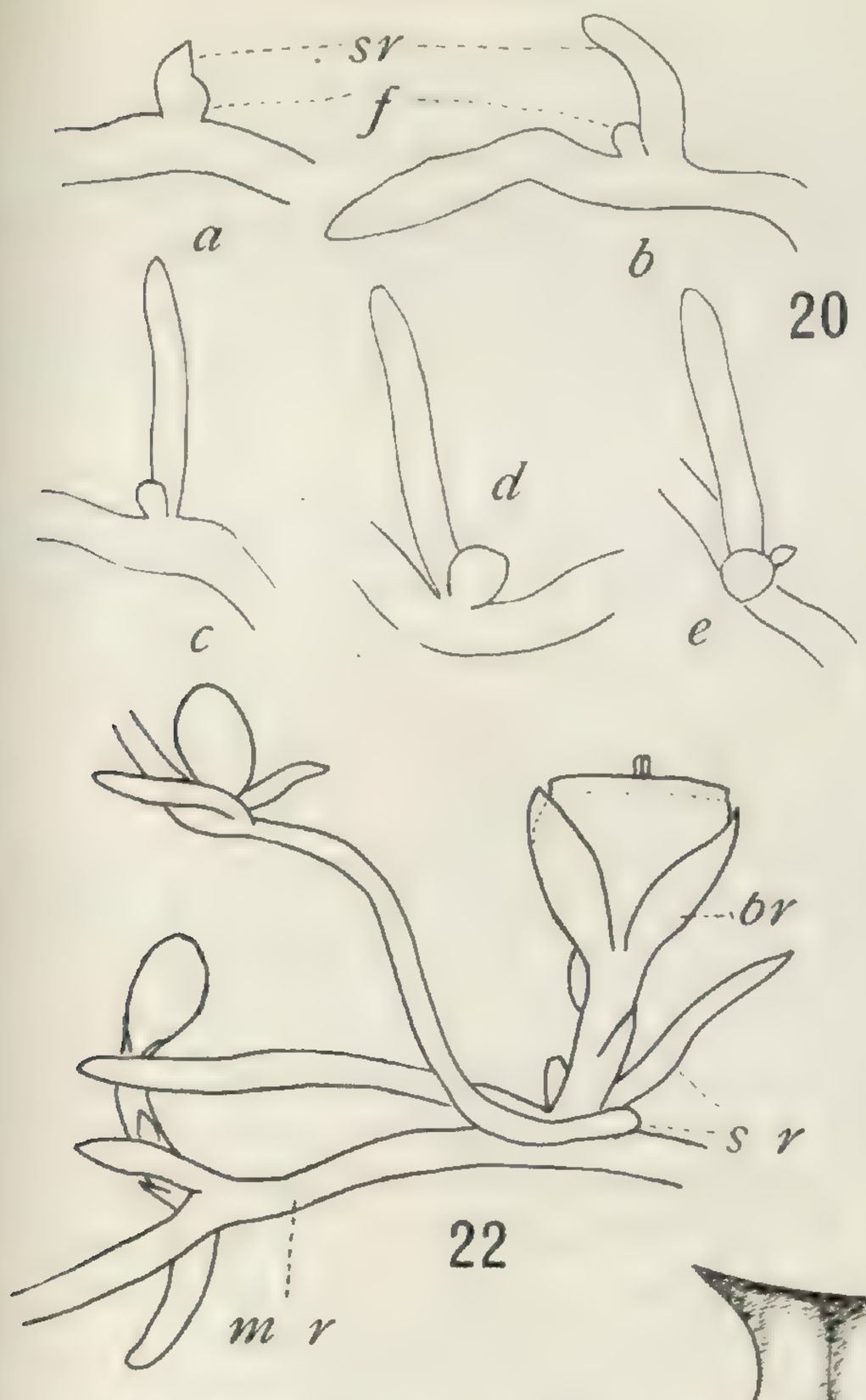

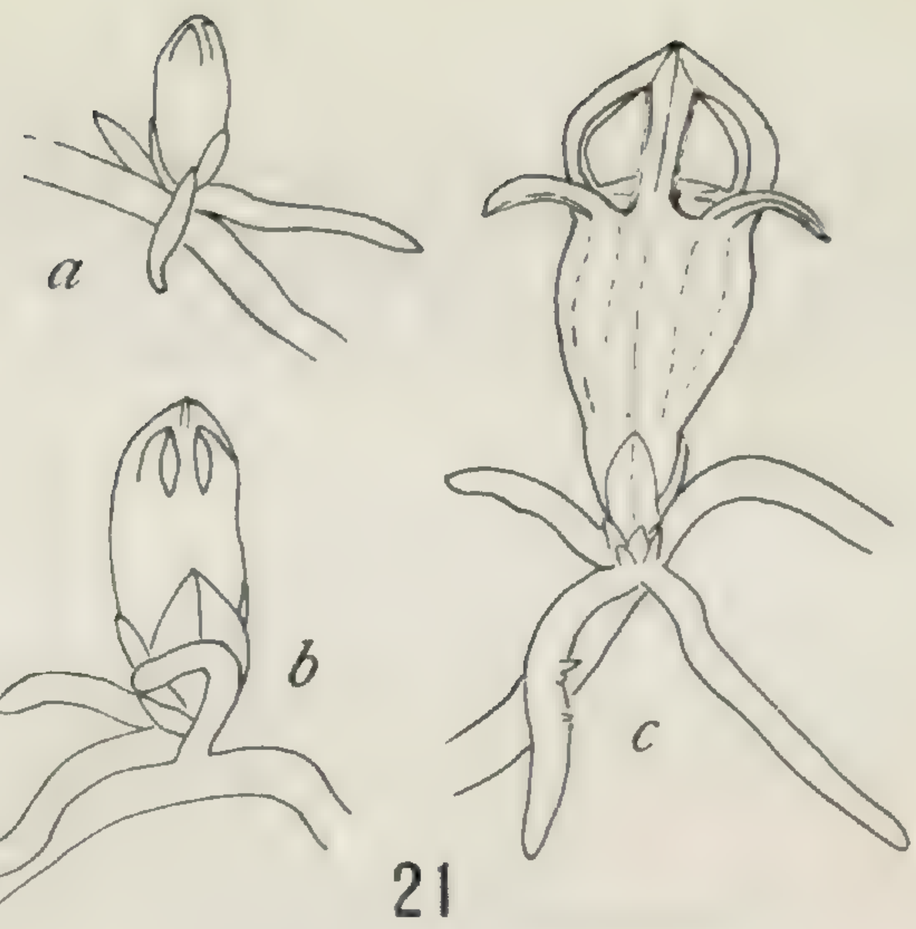

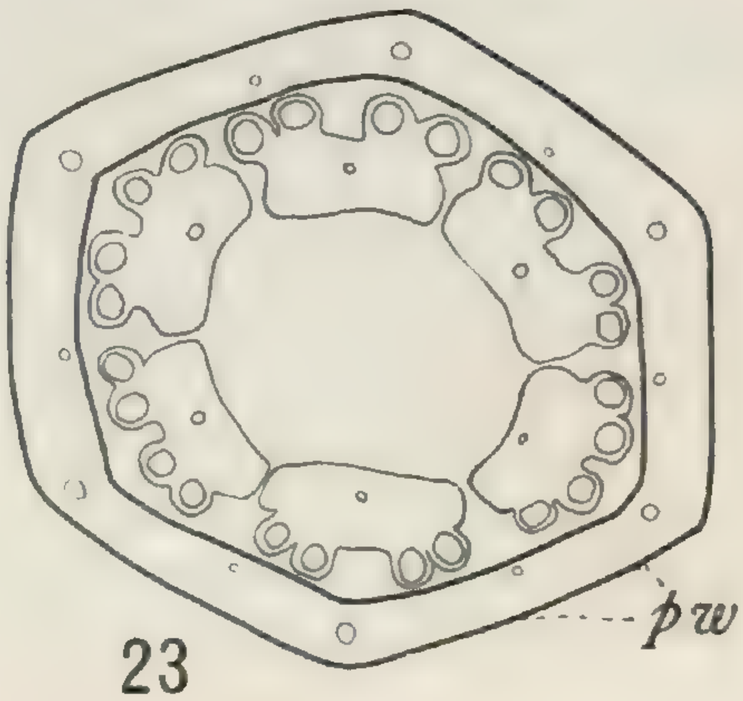

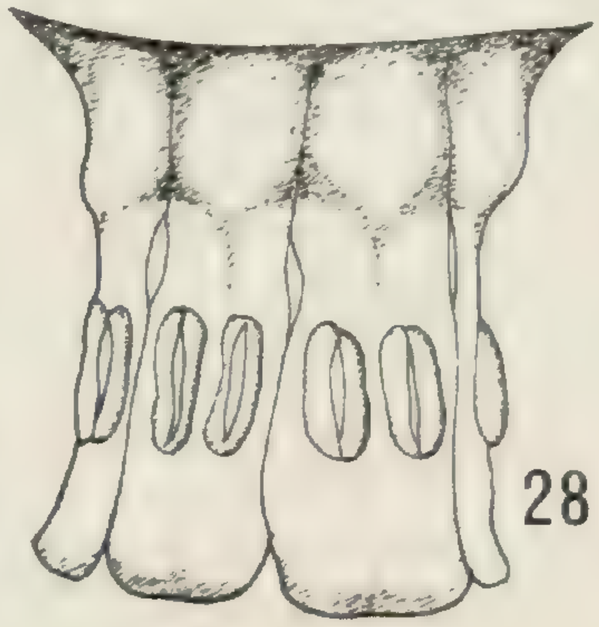

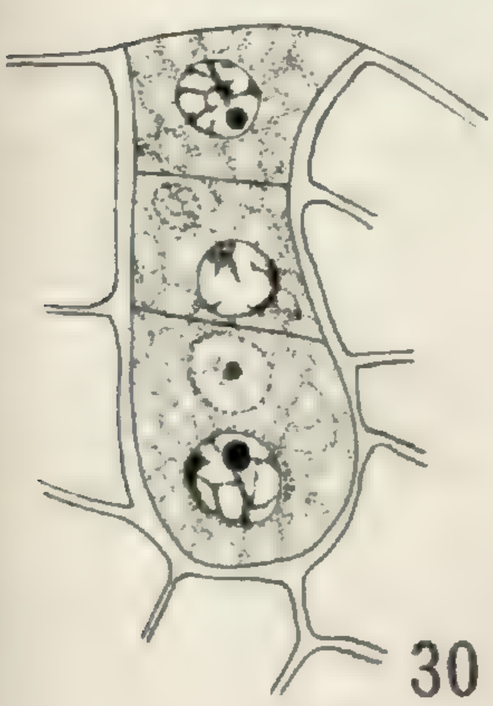

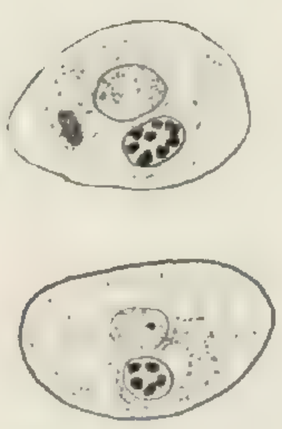

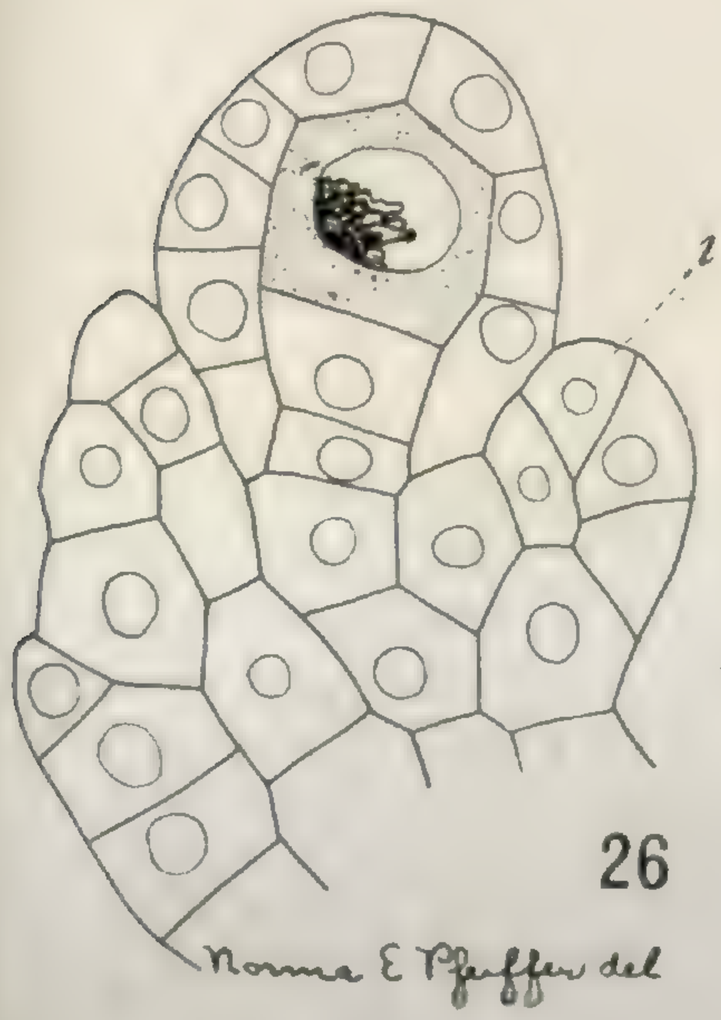

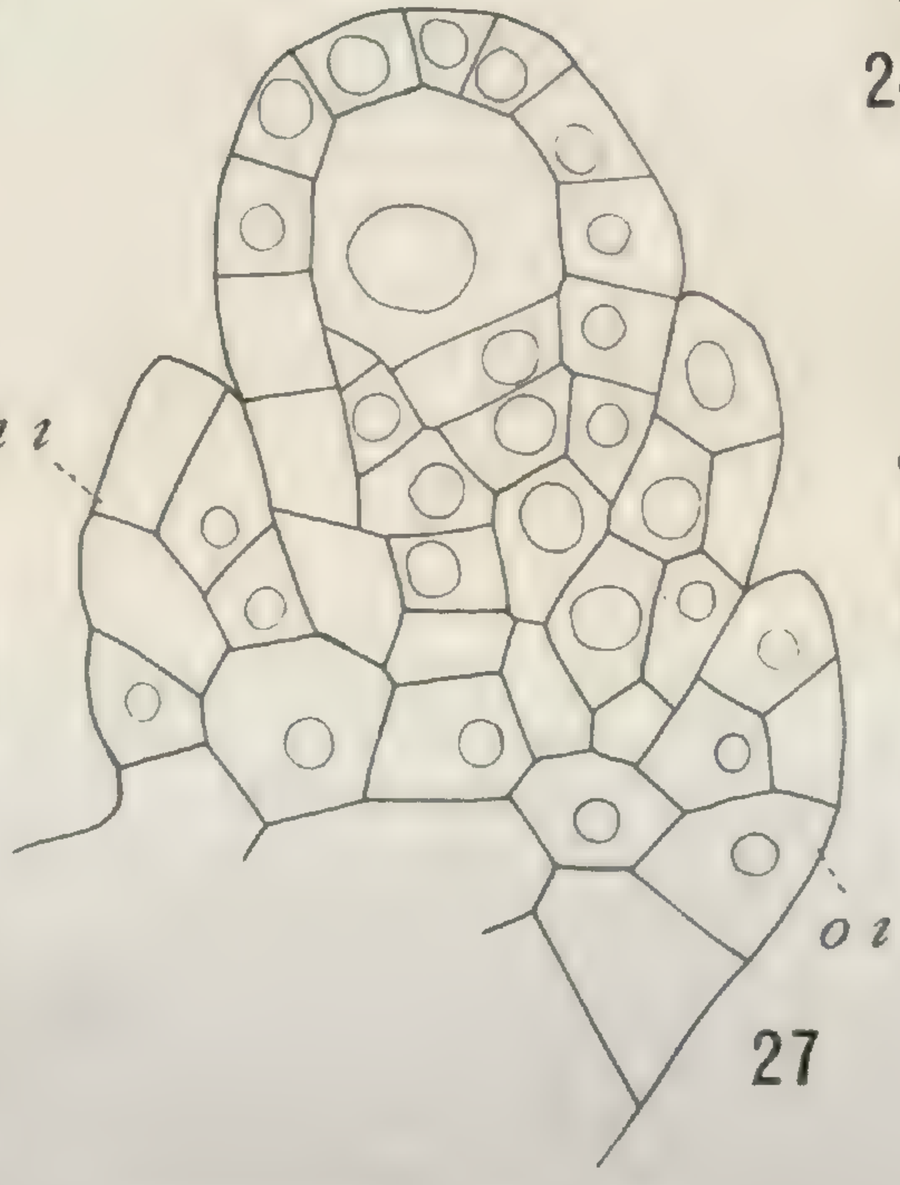

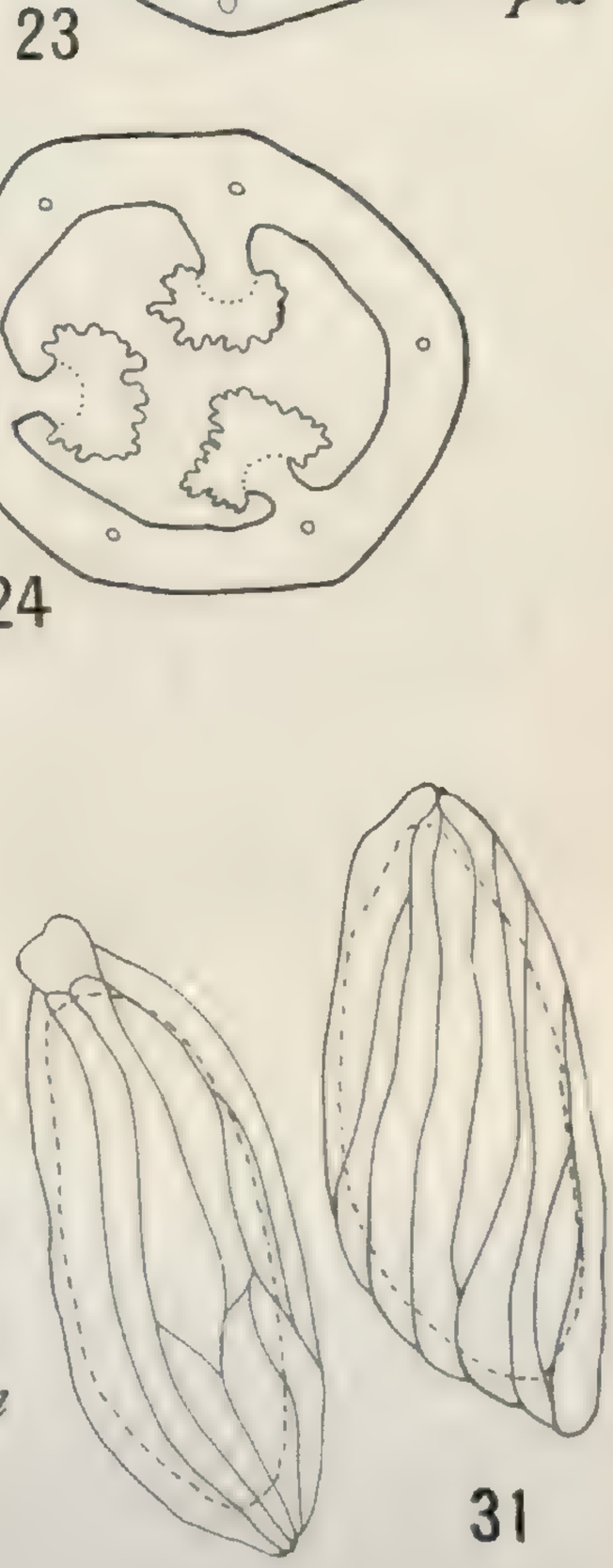

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FIG. 12.—Longitudinal section of tip of root, with young bud just developing; its proximity to the root cap is striking; ×52.

FIG. 13.—Older bud, in which the floral axis and secondary root tip have begun to differentiate; outlined portion in detail in fig. 19; ×26.

FIG. 14.—Cross-section of main root with secondary root; ×52.

FIG. 15.—Same main root, showing floral axis in neighboring section; ×52.

FIG. 16.—Later stage, where secondary root has elongated more rapidly than floral axis; longitudinal section of main root; ×52.

FIG 17.—Cross-section of main root, longitudinal of floral axis, at base of which second secondary root is developing; ×52.

FIG. 18.—Young flower in longitudinal section, showing arrangement of parts; ×52.

FIG. 19.—Detailed drawing (see fig. 13) to show relation of rudiments in bud; ×275.

FIG. 20.—Early stages in development of root and floral axis, as seen in habit material; *e*, same plant as *d* seen from above; ×4.

FIG. 21.—Subsequent stages; ×2.

FIG. 22.—Later stage, showing fruit; other floral axes arising on primary root and on secondary root.

FIG. 23.—Cross-section of young flower, showing stamens; ×26.

FIG. 24.—Cross-section of same flower through ovary; ×26.

FIG. 25.—Cross-section of style of same flower; ×26.

FIGS. 26, 27.—Ovules at megaspore mother cell stage; ×835.

FIG. 28.—Stamen tube removed from flower, as seen from side toward perianth wall; the pollen sacs have dehisced longitudinally; ×8.

FIG. 29.—Microspores before shedding; the generative cell about to divide; remains of a prothallial cell(?) in one; ×835.

FIG. 30.—Embryo imbedded in endosperm; ×835.

FIG. 31.—Seeds at maturity; ×87.

CONCERNING THE PRESENCE OF DIASTASE IN CERTAIN RED ALGAE

E. T. Bartholomew

Introduction

In most starch-forming plants the starch grains are deposited in the plastids. The work of both early and recent investigators shows, however, that this is probably never true for the red algae. In this group of plants the grains are described as being formed in the cytoplasm, outside the plastids and often apparently quite independent of them.

In the red algae the grains, which resemble the starch grains of higher plants, do not usually give the customary blue color when treated with iodine or zinc chloriodide. In a few species, for example *Lorencia* sp., *Polysiphonia nigrescens*, and *Ceramium tenuissimum*,[1] the color with iodine varies from violet to almost blue, but in most species it ranges from light brown to wine red. There is such a wide divergence of reaction in the different forms tested that Oltmanns[2] suggests the possibility that each species will be found to give its own characteristic color reaction.

Saiki[3] found that by applying strong solutions of malt extract to commercial food preparations made from various red algae, such as *Gelidium* sp., *Porphyra* sp., and *Chondrus crispus*, no digestion whatever of the carbohydrates took place. From this and other experiments he concludes that the polysaccharid carbohydrates in these algal food preparations are not readily transformed into sugar by carbohydrate-digesting enzymes of animal origin, and scarcely more so by vegetable enzymes or bacteria.

König and Bettels[4] hydrolyzed, with acids, the carbohydrates in *Porphyra* sp., *Gelidium* sp., and commercial compounds com-

[1] Oltmanns, F., Morphologie und Biologie der Algen **2**:148.

[2] *Ibid.*

[3] Saiki, T., The digestibility and utilization of some polysaccharid carbohydrates derived from the lichens and red algae. Jour. Biol. Chem. **2**:251–265. 1906.

[4] König, J., and Bettels, J., Die Kohlenhydrate der Meeresalgen und daraus hergestellter Erzeugnisse. Zeitschr. Untersuch. Nahrungs- und Genussmittel **10**: 457. 1905.

posed of red algae. Their results showed the presence of galactose, fructose, and glucose in varying quantities. In carrageen (*Chondrus crispus*), MÜTHER and TOLLENS[5] found galactose and probably other hexoses belonging to the fructose and glucose groups.

The grains in the red algae often show the physical characteristics of the common starch grains of green plants as to hilum, striation, and effect of polarized light, but their place of deposition, their behavior toward iodine and zinc chloriodide, their sugar extracts, and their apparent resistance to the action of malt extract would tend to prove that they are not true starch. The grains appear and disappear at various times during the life of the plant, thus suggesting the presence of an enzyme which is an active agent in bringing about their decomposition.

The following experiments were performed that further evidence might be gained as to the nature of the substance composing the starchlike grains in the red algae, by determining whether or not the diastase that acts on the grains in these plants will also digest the common starch isolated from the higher plants. So far as is known to the writer, this is the first attempt in this direction, and a preliminary statement of the results is herewith recorded.

Material and methods

I. SPECIES FROM WHICH EXTRACTS WERE MADE

Polysiphonia variegata Ag., *Dasya elegans* Ag., *Agardhiella tenera* (J. Ag.) Schmitz, and *Ceramium*[6] lent themselves most readily to experimentation because of their abundance and the ease with which they could be obtained. *Grinnellia americana* Harv., *Griffithsia globifera* J. Ag., and *Chondrus crispus* (L.) Stack. were also used and tended to show the same results as those obtained from the foregoing species, but sufficient quantities of the latter named plants could not be procured at the time of these experiments to warrant the drawing of definite conclusions.

[5] MÜTHER, A., and TOLLENS, B., Ber. Deutsch. Chem. Gesells. **37**: 298–305, 306–311. 1904.

[6] Several species were used in the same mass of material.

2. METHOD OF EXTRACTION AND AMOUNTS OF FINAL PRECIPITATES

The extracts were made according to modified Buchner and Litner methods. Fresh vigorous algae were taken from the sea water and quickly rinsed under the tap. The excess tap water was removed by filter paper or cheese cloth. The algal material was then immersed in 95 per cent alcohol for 20 minutes. Having been freed from excess alcohol, it was next plunged into acetone for 10 minutes. The excess of this solution having been removed, the material was placed in fresh acetone for two minutes, partially dried by suction, and placed in an oven at 35°–40° C. until dry. Upon being taken from the oven, the dried material was cooled in a desiccator, thoroughly pulverized, placed in three times its amount of 20 per cent alcohol, and left standing 18–24 hours. At the end of this period the substance was filtered by suction through a Buchner funnel lined with cheese cloth and filter paper (it was necessary to filter the material by suction because of its viscosity), and to the liquid thus drawn off was added two and one-half times its volume of 95 per cent alcohol. After being filtered as above, the precipitate was washed in equal parts of ether and absolute alcohol, dried in a desiccator, and after being pulverized the extract was ready for use. The extraction treatment did not remove all of the color, so that the precipitates varied from brownish to light red.

Because of the difficulty experienced in filtering, the final precipitates were probably far from pure, but the following figures will show the amounts of precipitates obtained from the different species:

Polysiphonia variegata	21.00	grams	powder*	—0.15	gram	final ppt.
Ceramium	51.00	"	"	—0.15	"	" "
Dasya elegans	53.03	"	"	—0.06	"	" "
Agardhiella tenera	53.465	"	"	—0.23	"	" "
Agardhiella tenera	17.00	"	"	—0.106	"	" "

*Obtained after material had been dehydrated, desiccated, and pulverized.

3. ALGAL EXTRACTS AND COMMERCIAL DIASTASE

Solutions of various concentrations were made from the algal extracts, but the one giving the best results consisted of 0.03 gm. of extract dissolved in 5 cc. of distilled water. Owing to the

nature of the extracts, solutions of this concentration were quite viscous. In most cases the viscosity would about equal that of a 75 per cent solution of glycerine and water. That a check might be had as to the time required for action and as to the final results, solutions of commercial diastase were also used. The solutions were of the same concentration as those of the algal extracts and were applied and tested in the same manner in every case. Both Eimer and Amend's and Merck's diastase were used, with no apparent difference in the results.

4. STARCH PASTE

Corn starch was used in all experiments. The paste used was made by adding 0.25 gm. of starch to 100 cc. of water and boiling 6 minutes. The boiling, though done slowly and in an Erlenmeyer flask plugged with cotton, probably caused 10 per cent of the water to be vaporized. No water was added to make up for this loss. New solutions of paste were made for each series of experiments.

5. CONTROL MEASURES

To determine as far as possible all sources of error, the following control measures were taken:

a) The "A, B, and C" parts of Fehling's solution were mixed just preceding each series of experiments.

b) Tubes of untreated paste were tested each time and in the same manner as those tubes containing the paste plus the extracts.

c) To detect any error due to the presence of an active agent in the distilled water, tubes of the paste were treated each time with distilled water only and tested in the regular manner.

d) Toluol was used as a sterilizing medium, except in the Van Tieghem tests, in which 10 per cent alcohol was used.

e) All containers and instruments were carefully sterilized with a strong solution of potassium bichromate and sulphuric acid.

6. TEST METHODS

A. *Digestion in test tubes*

The proportionate amounts of starch, algal extracts, commercial diastase, and distilled water used in each series of tests were as follows:

Test tube no.	1–5 cc.	starch paste	plus	1 cc.	algal extract			
" " "	2–5 cc.	"	"	"	1 cc.	"	"	
" " "	3–5 cc.	"	"	"	1 cc.	commercial	diastase	
" " "	4–5 cc.	"	"	"	2 cc.	"	"	
" " "	5–5 cc.	"	"	"	1 cc.	distilled	water	
" " "	6–5 cc.	"	"	"	2 cc.	"	"	
" " "	7–5 cc.	untreated	starch	paste				
" " "	8–2 cc.	algal	extract	solution				
" " "	9–2 cc.	commercial	diastase	solution				

When the experiments were to run for a longer period, the amounts were increased, but the same proportions were maintained. At the end of certain periods, indicated in the tables, 2 cc. of the solution were taken from tube number one and placed in two smaller tubes, 1 cc. in each tube. This process was repeated until test samples had been taken from each of the 7 tubes. One of each pair of small tubes was treated with iodine and the other with Fehling's solution. The experiments were all kept at room temperature, which was rather low, the daily average being 18°.6 C.

B. *Digestion in Van Tieghem cells*

To determine microscopically the effect of the algal extract upon unboiled starch, small quantities of dry corn starch were placed in four Van Tieghem cells. Three of these were treated with extracts from *Polysiphonia*, *Agardhiella*, and *Ceramium*, the fourth with distilled water to act as a control.

7. TERMS USED IN TABLES TO DESIGNATE COLOR REACTIONS AND AMOUNTS OF PRECIPITATES

That the standard for the designation of iodine color reactions and for the amounts of copper precipitate might be as nearly as possible the same for each series of experiments, two sets of terms were adopted. These may be interpreted in the following manner by referring to PRANG'S *Standard of colors* (1898):

IODINE COLOR REACTIONS

As specified in tables	As illustrated by chart
1. Blue	Plate I, 1–B
2. Tinge of purple	" I, 1–BV
3. Blue-purple	" II, 1–BV
4. Purple	" II, 1–V
5. Light purple	" II, 2–V
6. Very light purple	" II, 3–V
7. Purple-brown	" V, 5–O
8. Brown	" III, 3–OYO

*Fehling's solution precipitates**

As specified in tables	Algal extract	Commercial diastase
1. Trace	Plate II, 6–OYO	Plate II, 6–YYG
2. Very slight	" II, 5–O	" II, 4–Y
3. Slight	" I, 6–OYO	" II, 2–Y
4. Small	" I, 5–O	" I, 4–Y
5. Fair	" I, 4–ORO	" I, 3–Y
6. Medium	" I, 3–ORO	" I, 2–Y
7. Marked	" I, 3–RO	" I, 1–Y
8. Very marked	" I, 2–RO	" I, Y

*Although the precipitates from the two different extracts were of different colors, the terms used designate, as nearly as could be estimated, equal amounts of the two precipitates.

These designations were of course only relative, but they served very well as a guide. The colors designated in the tables were recorded within a few minutes after the application of the testing reagents.

Results

I. DIGESTION IN TEST TUBES

Tables I–IV were chosen promiscuously from a large number of tables, and are used as types to show the effect of the algal extract on starch paste and its rate of action in comparison with that of commercial diastase.

TABLE I

EXTRACT FROM CERAMIUM

Amounts of various solutions	After 11 hours		After 24 hours	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Tinge of purple	Trace	Blue-purple	Slight
2 cc. algal extract+5 cc. starch paste	Blue-purple	Slight	Purple	Small
1 cc. commercial diastase+5 cc. starch paste	Light purple	Fair	Light purple	Medium
2 cc. commercial diastase+5 cc. starch paste	Very light purple	Fair	Light brown	Marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commercial diastase	.....	Medium	.....	Medium

The action of the algal extract is very slow when compared with that of commercial diastase, as is shown in the preceding tables I–IV. To determine whether the action of the algal extract would

TABLE II

EXTRACTS FROM POLYSIPHONIA VARIEGATA

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 12 HOURS		AFTER 36 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Tinge of purple	Slight	Light purple	Medium
2 cc. algal extract+5 cc. starch paste	Blue-purple	Small	Very light purple	Medium
1 cc. commercial diastase+5 cc. starch paste	Purple-brown	Marked	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Brown	Marked	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commercial diastase	.....	Medium	.....	Medium

TABLE III

EXTRACTS FROM AGARDHIELLA TENERA

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 16 HOURS		AFTER 28 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Blue	None	Tinge of purple	Trace
2 cc. algal extract+5 cc. starch paste	Tinge of purple	Trace	Blue-purple	Slight
1 cc. commercial diastase+5 cc. starch paste	Purple-brown	Medium	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Brown	Medium	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commercial diastase	.....	Medium	.....	Medium

cease before digestion was complete, experiments were set up as before but allowed to run for a longer time. The results are shown in table V.

TABLE IV

EXTRACT FROM DASYA ELEGANS

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 17 HOURS		AFTER 25 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Blue	None	Blue ?	Trace
2 cc. algal extract+5 cc. starch paste	Blue ?	Trace	Tinge of purple	Very slight
1 cc. commerical diastase+5 cc. starch paste	Very light purple	Marked	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Purple-brown	Marked	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commerical diastase	.....	Medium	.....	Medium

TABLE V

EFFECTS OF LONGER CONTINUED ACTION OF THE ALGAL EXTRACTS AND THEIR COMPARATIVE RATES OF ACTION

AMOUNTS OF SOLUTION ON 5 CC. OF STARCH PASTE	AFTER 3 DAYS		AFTER 6 DAYS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
2 cc. Agardhiella extract	Purple	Fair	Very light purple	Very marked
3 cc. Agardhiella extract	Light purple	Medium	Very light purple	Very marked
2 cc. Ceramium* extract	Tinge of purple	Very slight	Blue-purple	Slight
3 cc. Ceramium* extract	Blue-purple	Slight	Purple	Fair
2 cc. Polysiphonia extract	Blue-purple	Medium	Very light purple	Very marked
3 cc. Polysiphonia extract	Purple	Marked	Very light purple	Very marked
2 cc. distilled water	Blue	None	Blue	None
3 cc. distilled water	Blue	None	Blue	None
Control, 10 cc. starch paste	Blue	None	Blue	None

*It took nine days for *Ceramium* to complete digestion.

2. DIGESTION IN VAN TIEGHEM CELLS

The cells were examined daily and it was found that in those containing the starch treated with the algal extracts, very noticeable corrosion of the grains was taking place. The rates of digestion agreed very well with the tabulated results in table V, *Polysiphonia* and *Agardhiella* acting more rapidly than *Ceramium*. The results thus obtained in the first trial were confirmed by repeating the experiment several times. Marked acceleration of corrosion was obtained by placing the cells in a warming oven at a temperature of 26° C. No corrosion could be detected in the control cells.

Discussion

That the disappearance of the starchlike grains in the living tissues of the red algae is due to the presence of some catalytic agent, which may be extracted and applied to corn starch and its derivatives with positive effects, is demonstrated by the results recorded in the foregoing tables. The action of this extract when applied to starch paste is very similar to that of commercial diastase. This is shown by the fact that in both cases the digestion of the starch appeared to be a series of processes leading more or less gradually from the amyloses down through the dextrins until amylolytic action was complete. This is brought out clearly not only by variations in color, but also by the fact that often when the iodine would indicate a marked degree of change in the solution, still the Fehling's test would show the presence of but very little sugar. It would appear from this that the algal extract, like the diastase of the higher plants, is not one simple enzymè but a complex of amylases and dextrinases. Then, if we may argue from analogy, the grains in the red algae, commonly referred to as starch, are probably, as MEYER[7] and others suggest, a combination of both starch and dextrins, or, as BÜTSCHLI[8] thinks, possibly a transitional stage between amyloporphyrin and amyloerythrin.

No direct evidence was obtained by experimentation as to whether or not this carbohydrate-digesting enzyme of the red algae

[7] MEYER, A., Untersuchungen über die Stärkekörner. Jena. 1895.

[8] BÜTSCHLI, O., Notiz über d. sogen. Florideen Stärke. Verh. Naturf. Med. Ver. Heidelberg N.F. 7:519–528. 1903.

might also act as a synthetic agent in forming the grains in question. BELZUNG[9] found that in some species the younger the grains the more nearly they came to giving the normal blue color reaction when treated with iodine. Hence, if this enzyme does act synthetically, it appears that, in some cases at least, the action is just the reverse of that which takes place in some of the higher plants, for example, in *Pisum sativum*, in which the erythrodextrins are formed first, the amylases not appearing until the grains are almost mature.

The difference in rate of amylolytic action between the algal extract and the commercial diastase is very noticeable. This is already indicated in tables I–IV. This variation may have been due to difference in concentration. The algal extracts were known to be far from pure, and it was for this reason that solutions of rather high concentration were applied to the starch paste. Table V indicates that there is also considerable dissimilarity in digestive power between the algal extracts themselves. Here again is the possibility that this may be attributed to an inequality in purity, for the extracts did not all present the same degree of difficulty in filtration. However, the explanation is hardly to be looked for in this direction, for although *Agardhiella* and *Polysiphonia* extracts were more difficult to filter than was the *Ceramium* extract, yet the two former completed amylolysis in 6 days, while the latter required 9 days under the same conditions, as is shown in table V.

As a result of recent experiments, ACHALME and BRESSON[10] maintain that diffusion is an important factor in the rate of enzymatic action. They found that an increase in viscosity of the solution meant a like decrease in the rate of action of invertase upon saccharose. They also maintain that this is true for emulsin, trypsin, and for organic oxidases. As has been said, the viscosity of the algal extracts would about equal a 75 per cent solution of glycerine in water. That this condition influenced the amylolytic action

[9] BELZUNG, E., Recherches morphologiques et physiologiques sur l'amidon et les grains de chlorophylle. Ann. Sci. Nat. Bot. VII. **5**:223–228. 1887.

[10] ALCHALME, P., and BRESSON, M., Influence de la viscosité du milieu sur les actions-diastasiques. Compt. Rend. **152**:1328–1330. 1911; also Du rôle de la viscosité dans les variations de l'action de l'invertine suivant les concentrations en saccharose. Compt. Rend. **152**:1420–1422. 1911.

of the extracts is not evident. It is true that the viscosity of the algal extracts greatly exceeded that of the commercial diastase and that action was very much more rapid in the latter than in the former; but this may have been due to some other factor, for although the extract from *Polysiphonia* was quite noticeably more viscous than that from *Ceramium*, yet the former completed digestion in two-thirds of the time required by the latter. Here, as before, we cannot draw definite conclusion, for there may have been a marked variation in purity between the two extracts. In some of the higher plants we find diastases that work very slowly, and it is not impossible that the algal diastases are also of this nature. It is a well known fact that by the addition of very small amounts of such substances as free mineral acids, neutral phosphoric acid compounds, salts of aluminium, and asparagin salts, the amylolytic action of the diastase from higher plants is very much accelerated, and it might be mentioned here that small amounts of aluminium acetate and sodium chloride had the same accelerating effect upon the algal extracts.

The Van Tieghem tests were of interest not only because they corroborated the macrochemical tests, but also because they showed that the manner of attack of the algal extracts was such that the starch grains were corroded. This would indicate that at least the most active enzyme present was a translocation rather than a secretion diastase.

The contrast between the colors of the precipitates of the different mediums tested with Fehling's solution was very marked. The precipitates in the tubes containing the paste treated with algal extract was of the usual "brick-red" color. Paste treated with commercial diastase gave a light yellow precipitate, even though the tubes were allowed to stand for a considerable length of time before the test was made. This would indicate the presence of some colloid which prevented the deposit of free copper oxide. The precipitate in the tubes containing the algal extract showed that whether or not the viscous nature of the extract retarded amylolytic action, there were no colloids or other substances present which prevented a free deposit of the copper oxide.

Conclusion and summary

1. There is present in the red algae a diastase which will digest the starch of higher plants.

2. The manner of action of this enzyme indicates that it is at least partially composed of a translocation diastase.

3. The diastase of the red algae, like that of the higher plants, is probably not composed of a single enzyme, but of a series of amylases and dextrinases.

4. Judging by the action of the algal extract upon corn starch, the diastase is a rather slow-working enzyme.

5. The series of digestion processes resulting from the application of the algal diastase to corn starch would indicate that the substance composing the grains of the red algae is very similar to that of the starch grains of higher plants.

These experiments were begun at Woods Hole, Massachusetts, and completed at the University of Wisconsin. The writer wishes to take this opportunity to extend his thanks to Professor B. M. DUGGAR, at whose suggestion and under whose direction the work was begun, and also to Professor R. A. HARPER and Professor W. G. MARQUETTE for valuable assistance and helpful suggestion.

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THE MALE GAMETOPHYTE OF ABIES

A. H. Hutchinson

(WITH FIFTEEN FIGURES)

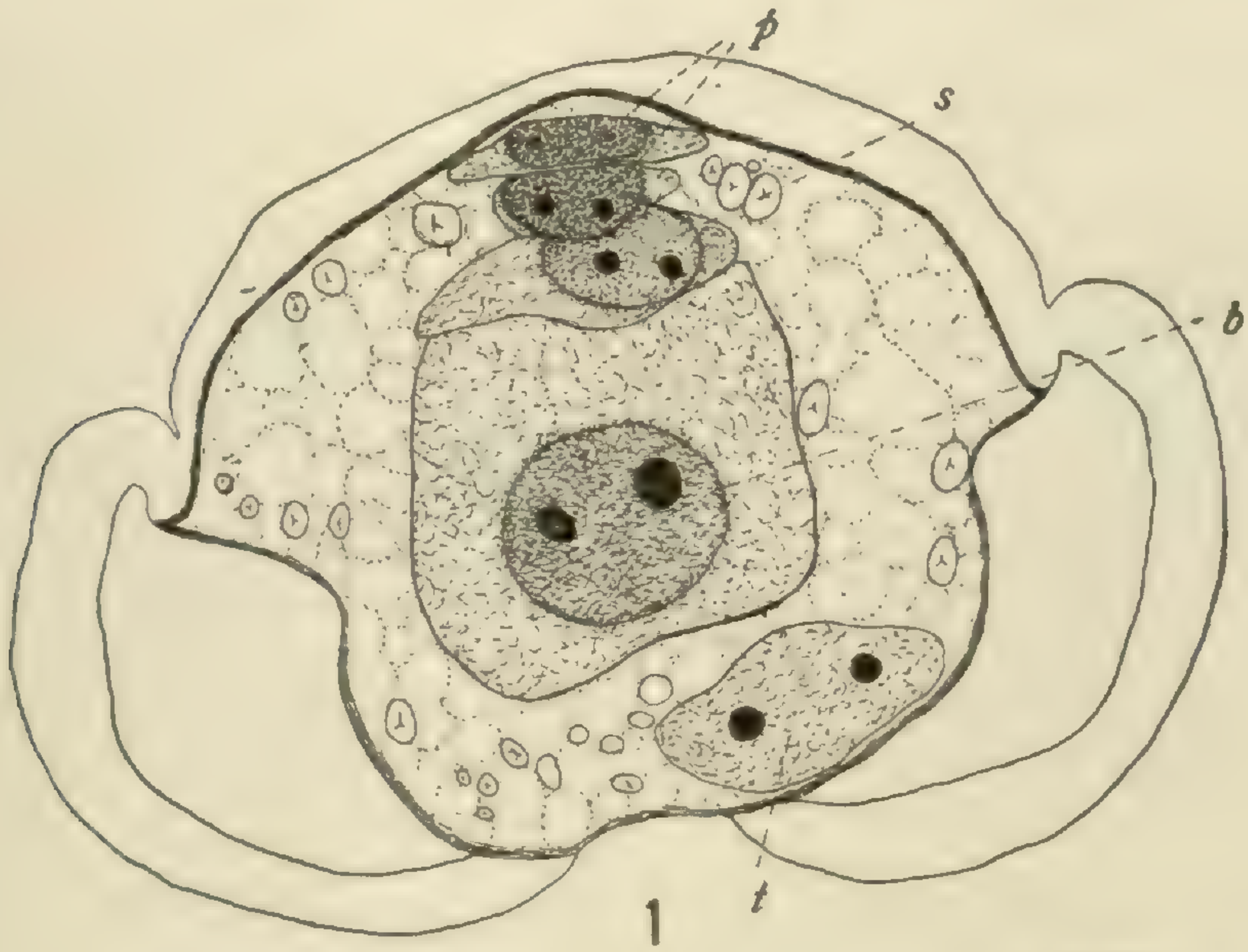
In a study of *Abies balsamea* as a type of the Coniferae, a number of irregularities in the contents of the pollen grain have been observed. Since the material used was collected from different trees and in different years, these irregularities cannot be regarded as abnormalities; and since further examination showed that similar conditions prevail in at least two other species of *Abies*, these observations have been thought worthy of record.

For the material I am indebted to Professor R. Wilson Smith. *A. balsamea* was collected in Ontario; *A. Veitchii* and *A. brachyphylla* were obtained through the courtesy of the authorities of the Royal Gardens at Kew.

The pollen grain of *Abies balsamea* is very large, its diameter being about twice that of *Pinus*. At the time of shedding the normal grain contains two prothallial cells, a stalk cell, a body cell, and a tube nucleus, imbedded in a vacuolated protoplasmic mass which contains numerous starch grains (fig. 1). The nucleus of the body cell is surrounded by dense protoplasm and a cell membrane. The tube nucleus is large and usually compressed; in some instances the pressure, resulting from the extraordinary growth of the cells of the gametophyte, is insufficient to force it from its polar position to a lateral one. The stalk cell is much smaller than the body cell, and quite frequently is compressed into a cavity at the prothallial end of the latter. In *A. Veitchii* and *A. brachyphylla* the prothallial cells are very much flattened at this stage; but in *A. balsamea* it is not uncommon to find both prothallial cells retaining the characteristic nuclear structure. The size and permanence, however, varies with the individual as well as with the species.

The material examined does not show stages earlier than the division of the central cell into generative cell and tube nucleus. Peculiarities in this division are described later. The periclinal

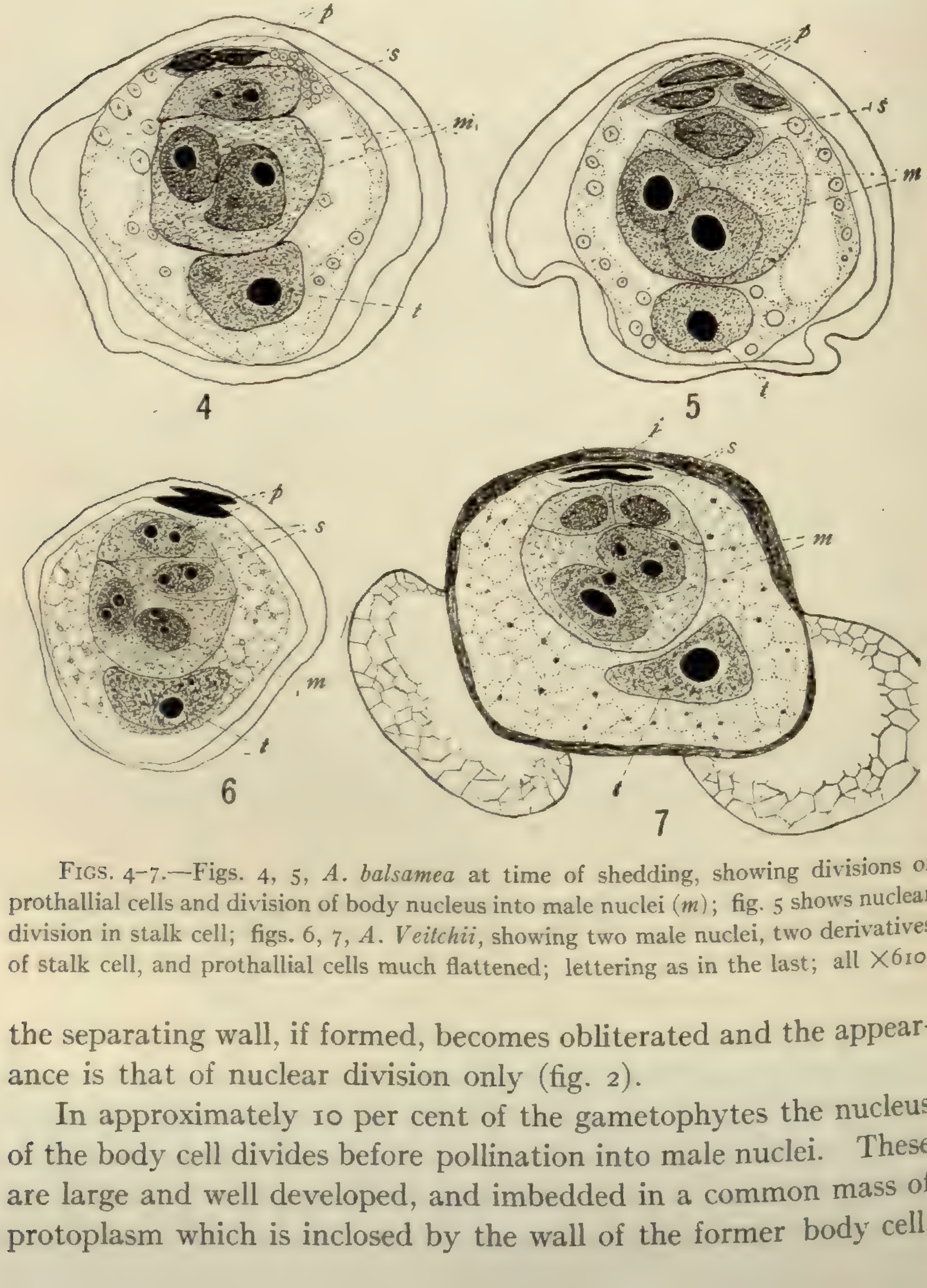
division into stalk and body cells takes place six or seven days before pollination. At this time the prothallial cells are at their maximum size.



FIGS. 1–3.—*Abies balsamea:* fig. 1, ripe pollen grain; figs. 2, 3, at time of shedding, showing divisions of prothallial cells; *p*, prothallial cells; *b*, body cell; *s*, stalk cell; *t*, tube nucleus; all ×610.

Frequent variations in the number of prothallial cells occur. The maximum number is four (figs. 2 and 4), but three are of more frequent occurrence (figs. 3 and 5). The division, as indicated by the position of the daughter cells, is anticlinal, either in or at right angles to the plane of the wings, hence they are best seen in a polar view. The presence of two derivatives of a prothallial cell, wher

they lie one over the other, is easily overlooked. It is difficult, therefore, to determine the proportion of pollen grains in which there are more than two. In *A. balsamea* about 8 per cent show a division of one or both of the original prothallial cells; in *A. Veitchii* and *A. brachyphylla* the number is smaller. In some cases



FIGS. 4–7.—Figs. 4, 5, *A. balsamea* at time of shedding, showing divisions of prothallial cells and division of body nucleus into male nuclei (*m*); fig. 5 shows nuclear division in stalk cell; figs. 6, 7, *A. Veitchii*, showing two male nuclei, two derivatives of stalk cell, and prothallial cells much flattened; lettering as in the last; all ×610.

the separating wall, if formed, becomes obliterated and the appearance is that of nuclear division only (fig. 2).

In approximately 10 per cent of the gametophytes the nucleus of the body cell divides before pollination into male nuclei. These are large and well developed, and imbedded in a common mass of protoplasm which is inclosed by the wall of the former body cell.

MIYAKE describes two male nuclei which enter the archegonium, and the one which fuses with the egg nucleus is said to be the larger. Before pollination, no difference in the size of structure could be detected (figs. 4 and 7), and MIYAKE's drawings show little or no difference in the pollen tube stage.

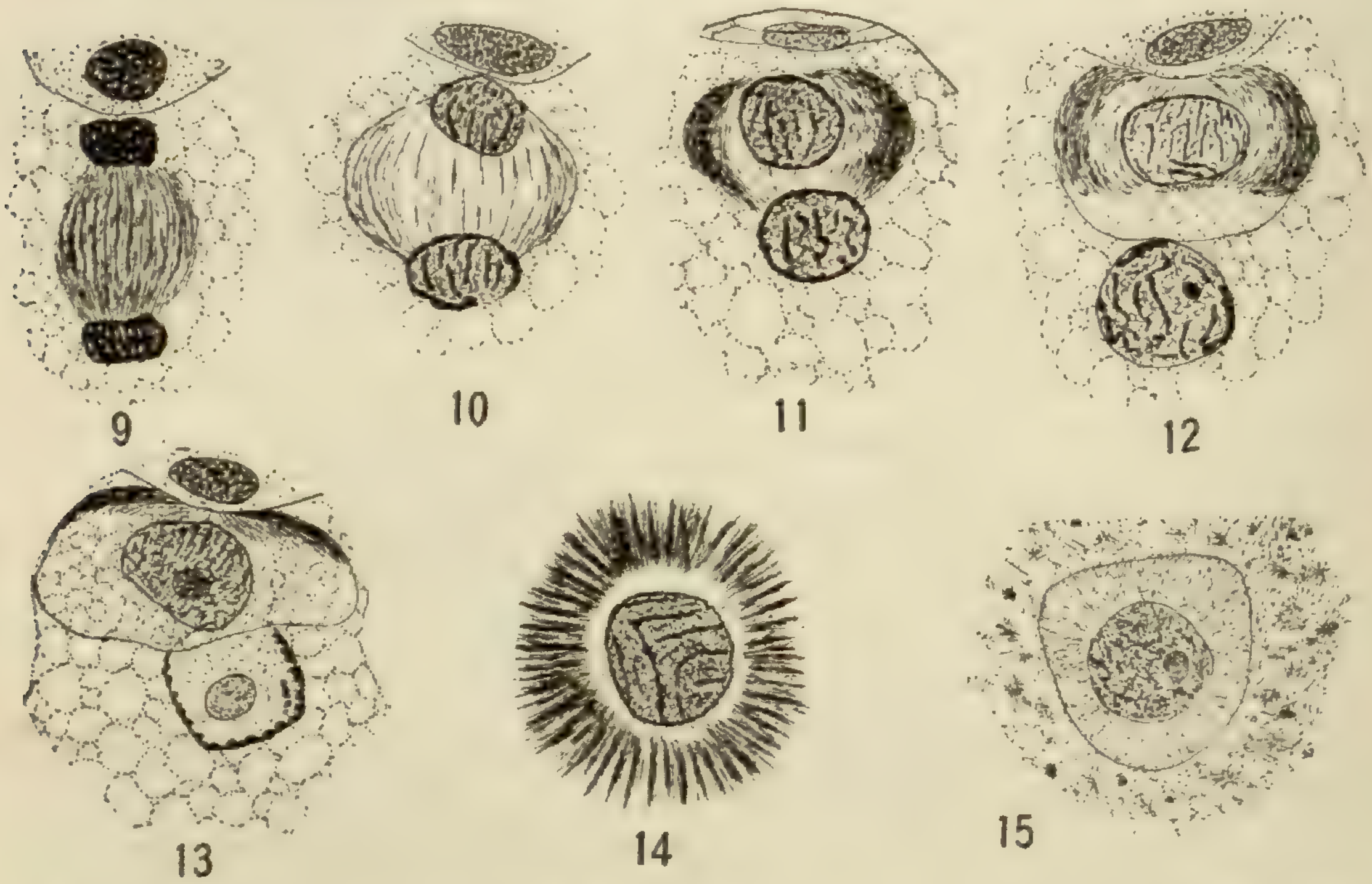
Occasionally the stalk cell also divides to form two derivative cells. The division is sometimes anticlinal (fig. 7), and sometimes periclinal (figs. 5 and 6). When this division takes place, the resulting nuclei frequently become uniformly granular and gradually degenerate. In this case also the separating walls are not always present (fig. 5) and the appearance is suggestive of amitosis.

There may be as many as four derivatives of the generative cell. In order to ascertain how many of the nuclei enter the egg at fertilization, an examination of the archegonium was made. Frequently there are four supernumerary nuclei at the micropylar end of archegonium after fertilization and during the proembryo stage (fig. 8). These in all probability are the second male nucleus, the two derivatives of the stalk cell, and the tube nucleus. MIYAKE states that these nuclei have the power of division. An examination of pollen grains lodged in the micropyle shows that while the other contents have passed into the pollen tube, the prothallial cells do not escape, but are retained by the intine in which they are imbedded.

FIG. 8.—*A. balsamea:* embryo sac with four supernumerary nuclei at micropylar end, and first four nuclei of proembryo; ×610.

The division of the generative cell in *Abies* may be an adaptation to the rapid succession of events between pollination and fertilization: about 4 weeks in *A. balsamea* and 13 weeks in *Pinus*. *Picea*

excelsa (POLLOCK) is similar in this respect. The division of the stalk cell cannot be so regarded; it may represent ancestral conditions. If so, it indicates a survival in *Abies* of a more ancient type of gametophyte than has been reported in any other of the Abietineae. Further, as the presence or absence of prothallial cells is a rather constant character of the different groups of Coniferae, and thus may have a phylogenetic significance, the presence of several prothallial cells may suggest a connection of the abietin-



FIGS. 9–15.—*A. balsamea* ten days before shedding: figs. 9–13, successive stages in telophase of mitosis of central cell; the relative positions are indicated by that of the prothallial cell; fig. 14, polar view of stage shown in fig. 13; fig. 15, cross-section of generative cell below central line during stage shown in fig. 13, or just after formation of cell membrane; all ×926.

ean and araucarian lines; which connection is also indicated by evidence accumulating from other sources.

Certain peculiarities in the division of the central cell to form the generative cell and tube nucleus have been mentioned. Attention was drawn to these by the very conspicuous radiating strands which surround the generative nucleus, as seen in a polar view (fig. 14). Further investigation shows these to be derived from the spindle, and that they take part in the formation of the cell membrane.

In the late telophase no well defined cell plate is formed, although thickenings of the fibers may occur (fig. 9). The central fibers of the spindle disappear, either ceasing to be differentiated by the stain, or, as is more probable, they move out and become peripheral (fig. 10). Gradually the spindle widens and moves away from the tube nucleus, thereby inclosing the generative cell in the form of a hollow globe (figs. 10 and 11). The fibers continue to move upward until they come in contact with the wall of the second prothallial cell (figs. 12 and 13). Meanwhile the cell membrane is formed in the position occupied by the peripheral fibers. When the wall is complete the fibers have disappeared. Although derived from the spindle, the cell wall does not originate from a cell plate, but from the fibers after they have surrounded the generative nucleus. Whether or not the fibers fuse laterally is difficult to determine; that they are very numerous and form a dense, almost continuous, envelope about the generative nucleus is shown in a polar view (fig. 14). The definite cell membrane which is formed is best shown in cross-section. Fig. 15 represents such a section of a cell at the stage shown in fig. 13.

These peculiarities may perhaps be accounted for by the fact that the division is internal, and only one of the resulting nuclei is inclosed by a wall; hence this method of delimiting the cellular protoplasm. One is reminded of the formation of the plasma membrane about the ascospore of *Phyllactinia* as described by HARPER.

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CURRENT LITERATURE

BOOK REVIEWS

Parasitic fungi

A book treating of the fungi which cause plant diseases, which limits itself to the diseases already known in the United States and to those which, known elsewhere, seem likely to invade this country, ought to be received gratefully by the American student of plant pathology. In his new book, STEVENS[1] gives keys and descriptions of the fungi which cause plant diseases and also of such saprophytic forms as are obviously closely related to the parasitic forms. But he does more than give a taxonomic account of the parasitic forms, since he presents the results of cytological work, usually with the original figures, in all those groups in which extensive cytological studies have appeared. As would be expected, he also goes into details of culture methods in such groups as the rusts, where an elaborate method of procedure has been evolved by specialists.

Besides the Introduction, which treats of the nature of fungi and their relation to other thallophytes, the book comprises three divisions. The first division treats of Myxomycetes, the second of Schizomycetes. There follows a bibliography of 210 titles relating to the Introduction, Myxomycetes, and Schizomycetes. The third division of the book, which treats of Eumycetes, is divided into a portion on Phycomycetes with a bibliography of 149 titles, one on Ascomycetes with 344 references, one on Basidiomycetes with 345 references, while the fourth portion on Fungi Imperfecti is followed by a list of 459 references. In addition, there are 64 books and 12 periodicals which are listed as "some of the most useful." In connection with so extensive a bibliography it is to be lamented that citations are not made in full so as to include the titles of papers. Such a list as STEVENS publishes, in which appears an author's name, a volume reference (abbreviated as much as possible), a first page, and a date, might be quite satisfactory had we reached the day of perfect proofreading, when each word and figure would stand as it should, and it would not be necessary to turn back to the index of the volume indicated to see whether it was merely the page reference that was wrong or whether the volume number was at fault. Nor can we say that up to this moment we have found the proofreading of these bibliographies imperfect, but a number of very obvious slips in the text would indicate that this might be possible. Thus, on page 72, *Cladochytrium*, after being listed in the key under *intracellular* forms, is described as a genus containing about ten *intercellular* parasites; and on page

[1] STEVENS, F. L., The fungi which cause plant diseases. 8vo. pp. viii+754. *figs. 449*. New York: The Macmillan Co. 1913. $4.00.

75, it is said of *Dictyuchus:* "This genus of the Saprolegniaceae contains the only parasite genus in the first two families." Turning then to the end of the book, we find sprodochium for sporodochium on page 664, "synnema or corymium" for *coremium* on page 565, while *synema* appears on page 694 of the glossary, and on page 443 Merasmieae appears instead of Marasmieae.

In several points STEVENS helps to overcome inconsistencies which are common in works on parasitic fungi. Striking among these is his listing of the three groups of Fungi Imperfecti (the group which is characterized chiefly by the imperfection of our knowledge of them) in the three orders Sphaeropsidales, Melanconiales, and Moniliales, rather than Sphaeropsidales, Melanconiales, and Hyphomycetes. In this connection it is unfortunate that he should speak of the types of *fructification* displayed by Fungi Imperfecti as pycnidia, acervuli, and hyphae, in spite of the fact that the definition of hypha in the glossary is "the thread-like *vegetative* part of a fungus."

Altogether this book, with its concise, clear keys of parasitic fungi, which aims to give at least one illustration as well as description of each genus of economic importance in the United States, and which has a comprehensive bibliography, must prove a stimulus to the student of plant pathology.—WANDA M. PFEIFFER.

NOTES FOR STUDENTS

Current taxonomic literature.—H. ANDRES (Verhandl. Bot. Ver. Prov. Brandbg. **54**:218–227. 1913) has published two species of *Pyrola*, one being from the state of Washington, and (Oesterreich. Bot. Zeitschr. **63**:68–75. 1913) three species are added from the Pacific Coast. The same author (Allg. Bot. Zeitschr. **19**:81–86. 1913) in a closing article on the Pyrolaceae includes the description of a new species (*P. cordata*) from Ontario, Canada.—H. H. BARTLETT (Rhodora **15**:81–85. 1913) in continuation of systematic studies on *Oenothera* has published jointly with G. F. ATKINSON two new species of this genus from New York.—O. BECCARI (Webbia **4**:143–240. 1913) under the title "Contributi alla conoscenza delle Palme" has published the results of further studies of the palms, describes a new genus (*Jubaeopsis*) from Central Africa, and gives a revision of the genus *Pritchardia* which includes several new species from the Hawaiian Islands.—G. BITTER (Rep. Sp. Nov. **12**:49–90, 136–162. *pls. 1, 2.* 1913) has published upward of 50 new species and several varieties of *Solanum* mostly from America.—S. F. BLAKE (Rhodora **15**:86–88. 1913) records two new forms of *Ophioglossum vulgatum* from eastern North America, and (*ibid.* 153–168) under the title of "Six weeks' botanizing in Vermont. I. Notes on the plants of the Burlington region" presents the results of a study of the plants collected in the Champlain Valley of Vermont in 1911, adding several species not hitherto recorded from the Burlington region and describes a number of new varieties and forms.—C. BÖRNER (Abh. Nat. Ver. Bremen **21**:245–282. 1913) under the title "Botanisch-systematische Notizen" has

proposed several new generic names for somewhat aberrant or habitally distinct forms of well known genera. The names are numerous and attention is called merely to their place of publication.—E. BRAINERD (Bull. Torr. Bot. Club **40**:249–260. *pls. 15–17.* 1913) describes four new hybrid violets; and (Rhodora **15**:106–111. *pl. 104.* 1913) in continuation of his studies on the violets presents a discussion on the Old World *Viola arenaria* DC. in relation to its American ally and characterizes a new variety, namely, *V. adunca* var. *glabra.* The same author (*ibid.* 112–115) under "Notes on new or rare violets of Northwestern America" describes a new variety of *V. cucullata* Ait. and adds several new hybrid combinations.—A. BRAND (Ann. Conserv. and Jard. Bot. Genève **15** and **16**:322–342. 1913) in continuation of studies on the Polemoniaceae records further information on this family and describes several new species and varieties from western United States, and (*ibid.* 343–344) publishes two new species of *Symplocos* from America.—T. S. BRANDEGEE (Univ. Calif. Pub. Botany **4**:375–388. 1913) has published 35 new species, based on collections made in Mexico by Dr. C. A. PURPUS in 1912.—N. L. BRITTON (Torreya **13**:215–217. 1913) describes 4 new species of Cyperaceae from the West Indies.—N. L. BRITTON and J. N. ROSE (Contr. U.S. Nat. Herb. **16**:239–242. *pls. 66–73.* 1913) in continuation of their studies in the Cactaceae have described from living specimens 7 new species from Mexico and Central America. The same authors (*ibid.* 255–262. *pls. 78–84*) under the title "The genus Epiphyllum and its allies" present a systematic treatment of *Epiphyllum*, to which *Phyllocactus* Link is referred as a synonym, and the immediately allied genera, as follows: *Epiphyllum* (28), *Disocactus* (2), *Zygocactus* (3), *Schlumbergera* (2), *Wittia* (3), and *Epiphyllanthus* (1); *Eccremocactus* and *Strophocactus*, each represented by a single species, are proposed as new generic types.—L. BUSCALIONI and G. MUSCATELLO (Malpighia **25**:187–250. 1912) under the title "Studio monografico sulle specie americane del gen. *Saurauia* Willd." give a synoptical revision of the genus and include descriptions of two new species from South America, and (*ibid.* 389–436. *pls. 7, 8*) in continuation of these studies add two more species of *Saurauia* from Mexico.—F. E. CLEMENTS, C. O. ROSENDAHL, and F. K. BUTTERS (Geol. and Nat. Hist. Surv. Minn., Minn. Bot. Studies. pp. ix+59. 1913) have issued the third edition of their "Guide to the spring flowers of Minnesota, field and garden."—O. F. COOK (Contr. U.S. Nat. Herb. **16**:243–254. *pls. 74–77.* 1913) presents a discussion of the false date palm (*Pseudophoenix Sargentii* Wendl.) and creates for it an independent family, namely *Pseudophoenicaceae.* In an accompanying key to the families of American palms the new family is placed between the *Ceroxylaceae* and the *Cocaceae.*—L. DAMAZIO (Broteria Ser. Bot. **11**:51–53. 1913) describes and illustrates a new species of *Cassia* (*C. itaculumiensis*) from Brazil.—H. DIEDICKE (Ann. Mycol. **11**:172–184. 1913) under the title "Die Leptostromaceen" describes two new genera, namely, *Pycnothyrium* found on leaves of *Mercurialis perennis* and *Thyriostroma* on *Pteris aquilina.*—A. D. E. ELMER (Leafl. Phil. Bot. **5**:1589–1750. 1913) in cooperation with several

specialists has issued articles 78–92 inclusive of the *Leaflets*. About 120 new species and varieties of Philippine plants are described.—F. FEDDE (Rep. Sp. Nov. **12**:278–279. 1913) records a new species and variety of *Corydalis* from Idaho.—M. L. FERNALD (Rhodora **15**:74–78, 92, 93, 168, 169. 1913) has published new varieties in *Bidens*, *Carex*, and *Betula*.—M. L. FERNALD and K. M. WIEGAND (*ibid.* 133, 134) characterize two new varieties of *Carex* from Newfoundland and (*ibid.* 135, 136) a new form of *Calamagrostis Pickeringii* Gray.—C. N. FORBES (Occ. Papers Bern. Pau. Bishop Mus. Eth. and Nat. Hist. **5**:3–26. 1913) places on record notes concerning the flora of the Hawaiian Islands and describes a new species of *Euphorbia* (*E. Stokesii*).—E. GADECEAU and O. STAPF (Rev. Hort. Paris **85**:422–426. 1913) describe and illustrate a new species of *Mandevillea* (*M. Tweedieana*) indigenous to South America.—L. GAIN (Deuxième Expédition Antarctique Française, 1908–1910, commandée par le J. CHARCOT, pp. 218. *pls. 1–8.* 1912) records the results of a study of the algae secured on the expedition and describes several species new to science.—H. A. GLEASON (Bull. Torr. Bot. Club **40**:305–332. 1913) in continuation of studies on the *Vernonieae* has published several new species and gives a synopsis of the group as represented in the West Indies.—A. GRIFFINI (Atti Soc. Ital. Sc. Nat. Milano **52**:61–104. 1913) under the title "Sopra alcuni Grillacridi e Stenopelmatidi della collezioni Pantel" includes the description of a new genus (*Paterdecolyus*) from India. The new genus is related to *Anabropsis*.—D. GRIFFITH (Monatsschrift für Kakteenkunde **23**:130–140. 1913) describes several new species of *Opuntia* and a new *Nopalea* from Southwestern United States and Mexico.—H. GROSS (Bull. Geogr. Bot. **23**:7–32. 1913) under the title "Remarques sur les Polygonées de l'Asie orientale" presents a synopsis of the genera of this family, as represented in eastern China, describes several new species and one new genus (*Pleuropteropyrum*).—H. E. HASSE (Contr. U.S. Nat. Herb. **17**:1–132. 1913) has published a "Lichen flora of Southern California." The region covered by the author is that portion of California south of the 36° parallel or about one-third of the state. Five families of lichens are recognized embracing 60 genera and approximately 360 species.—E. HASSLER (Rep. Sp. Nov. **12**:201, 202, 249–278. 1913) describes about 75 new species, belonging mostly to the Apocynaceae, Malvaceae, and Onagraceae, from Argentina and Paraguay. One new genus is characterized, namely, *Casimirella* of the Icacinaceae.—A. HEIMERL (Oesterr. Bot. Zeitschr. **63**:279–290. 1913) under the title "Die Nyctaginaceen-Gattungen *Calpidia* und *Rockia*" revives *Calpidia* Thour. and proposes a new genus (*Rockia*) based on *Pisonia sandwichensis* Hillebr. The same author (*ibid.* 353–356) has published a new species of *Selinocarpus* (*S. Purpusianus*) from Mexico.—A. A. HELLER (*Muhlenbergia* **9**:60–65. 1913) in an article entitled "Acmispon in California" recognizes 6 species of this genus, 4 of which are described as new to science. The same author (*ibid.* 67, 68) makes 23 new combinations in the Leguminosae, Onagraceae, and Ericaceae.—G. HINZE (Ber. Deutsch. Bot. Gesells. **31**:189–202. *pl. 9.* 1913) under the title "Beiträge zur Kenntnis der

farblosen Schwefelbakterien" proposes a new genus (*Thiovulum*) from the Gulf of Naples.—A. KORSCHIKOFF (*ibid.* 174–183. *pl. 8.* 1913) describes and illustrates a new genus (*Spermatozopsis*) of the Volvocales.—B. KOSO-POLJANSKY (Jour. Russe de Bot. no. 1–2. pp. 1–10. *pls. 1–5.* 1913) under the title "Species Umbelliferarum minus cognitae" has published several new species of Umbelliferae and includes a new genus (*Glochidopleurum*) based on *Bupleurum Sintenisii* Aschers. and Graeb.—F. KRÄNZLIN (Ann. K.K. Naturhist. Hofmus. Wien **27**:109–112. 1913) has published 5 new species of *Spiranthes* from South America.—K. KRAUSE (Smith. Misc. Coll. **61**: no. 16. p. 1. 1913) describes a new species of *Esenbeckia* (*E. Pittieri*) from Colombia.—G. KÜKENTHAL (Rep. Sp. Nov. **12**:91–95. 1913) under the title "Cyperaceae novae III" has published several new species and varieties including 4 from South America.—C. LAUTERBACH (Bot. Jahrb. **50**:1–170. 1913) in cooperation with certain specialists has issued the second article under the general title "Beiträge zur Flora von Papuasien." Several species new to science are recorded and the following new genera are proposed: *Mischocodon* Radlk. of the Sapindaceae, *Astelma* Schltr. of the Asclepiadaceae, *Ancylacanthus* and *Jadunia* Lindau of the Acanthaceae.—R. LAUTERBORN (Allg. Bot. Zeitschr. **19**:97–100. 1913) under the title "Zur Kenntnis einiger sapropelischer Schizomyceten" characterizes the following new genera: *Pelodictyon, Schmidlea, Pelogloea*, and *Peloploca*.—H. LÉVEILLÉ (Rep. Sp. Nov. **12**:99–103. 1913) has published several new species of flowering plants from Asia and includes a new genus (*Bodinieriella*) of the Ericaceae.—G. LINDAU (Ber. Deutsch. Bot. Gesells. **31**:243–248. *pl. 11.* 1913) publishes an account of a new fungus to which he gives the name *Medusomyces Gisevii.* The new genus is related to *Mycoderma*.—T. LOESENER (Rep. Sp. Nov. **12**:217–244. 1913) in cooperation with several specialists under the heading "Mexikanische und zentralamerikanische Novitäten IV" has published several new species of flowering plants.—Fr. MARIE-VICTORIN (Le Naturaliste Canadien **39**:177–189. 1913) under "Notes sur deux cas d'hybridisme naturel" treats *Nymphaea rubrodisca* (Morong) Greene as a hybrid between *N. americana* (Prov.) Miller and Standley and *N. microphylla* Pers., and records a new hybrid between *Lysimachia terrestris* (L.) B.S.P. and *L. thyrsiflora* L.—A. MAUBLAN (Bol. Minist. Agr. Ind. and Com. Rio de Janeiro **2**:126–130. 1913) in a paper entitled "Uma molestia do mamoeiro" describes and illustrates a new fungus (*Sphaerella Caricae*) found on leaves of the pawpaw (*Carica Papaya* L.) and proposes a new generic name (*Asperisporium*) based on *Fusicladium Peucedani* Ell. & Holw.—W. R. MAXON (Smiths. Misc. Coll. **61**: no. 4. pp. 1–5. *pls. 1, 2.* 1913) describes and illustrates a new genus of ferns (*Saffordia*) from Peru. The same author (Contr. U.S. Nat. Herb. **17**:133–179. *pls. 1–10.* 1913) under the title "Studies of tropical American ferns no. 4" has published a paper embodying results of a continued study of ferns and fern-allies, recording important data and describing new species in *Asplenium, Dicksonia, Odontosoria, Bommeria, Hemionitis, Lycopodium*, and *Cyathea*.—N. NAOUMOFF (Bull. Soc. Mycol.

France **29**:273–278. *pl. 13*. 1913) under the heading "Matériaux pour la flore mycologique de la Russie" includes the description of a new genus (*Rhodoseptoria*) found on leaves and fruit of *Prunus*.—B. NĚMEC (Rozpravy Ceské Akademie **21**:1–16. *pls. 1, 2*. 1912) describes and illustrates a new genus and species (*Anisomyxa Plantaginis*) parasitic on *Plantago lanceolata*.—J. A. NIEUWLAND (Am. Mid. Nat. **3**:85–91. *pl. 2*. 1913) has published a new species of violet (*Viola candidula*) from Michigan.—S. B. PARISH (Bot. Gaz. **55**:300–313. 1913) under the title of "California Paroselas" recognizes 9 species and several varieties of this genus indigenous to California. The same author (Muhlenbergia **9**:57–59. 1913) in an article entitled "Additions to the known flora of southern California" places on record important data concerning that flora and describes a new species of *Atriplex* (*A. saltonensis*) from the Colorado Desert.—F. W. PENNELL (Bull. Torr. Bot. Club **40**:401–439. 1913) gives a synoptical revision of the *Agalinanae*, recognizing four genera, namely *Macranthera*, *Afzelia*, *Aureolaria*, and *Agalinis*. Descriptions of several new species are included.—C. A. PICQUENARD (Trav. du Lab. de Concarneau **4**: fasc. 3. pp. 1–5. *pl. 1*. 1912) has proposed a new genus (*Guerinea*) based on *Hapalidium callithamnoides* Crouan.—R. PILGER (Bot. Jahrb. **50**:171–287. 1913) in continuation of monographic studies of the Plantaginaceae gives a detailed consideration of the section *Novorobis* of *Plantago*, recognizing 50 species of which nearly one-third are new to science. The same author (Rep. Sp. Nov. **12**:304–308. 1913) has published 6 new species of grasses from Patagonia and Tierra del Fuego.—L. QUEHL (Monats. für Kakteenkunde **22**:42. 1913) describes a new species of *Mamillaria* (*M. echinoidea*) from Mexico.—H. REHM (Ann. Mycol. **11**:166–171. 1913) characterizes a new ascomycetous genus (*Durandia*) based on *Sphaerographium Fraxini* (Peck) Sacc.—H. N. RIDLEY (Jour. Bot. **51**:201, 202. *pl. 527*. 1913) has published a new genus (*Aridarum*) of the Araceae from Borneo.— W. J. ROBINSON (Bull. Torr. Bot. Club **40**:193–228. *pls. 9–12*. 1913) under the title of "A taxonomic study of the Pteridophyta of the Hawaiian Islands III" presents a synoptical treatment of several genera and includes descriptions of new species in *Polypodium* and *Asplenium*.—R. A. ROLFE (Bot. Mag. *t. 8514*. 1913) describes and illustrates a new orchid (*Catasetum microglossum*) from Peru.—J. N. ROSE (Smiths. Misc. Coll. **61**: no. 12. pp. 1, 2. *pl. 1*. 1913) describes and illustrates a new poplar (*Populus MacDougalii*) from the Salton Basin, California.—P. A. RYDBERG (Bull. Torr. Bot. Club **40**:461–485. 1913) under the title "Studies on the Rocky Mountain flora XXIX" describes several new species of Sympetalae.—R. SCHLECHTER (Rep. Sp. Nov. **12**:104–109, 202–206, 212–216. 1913) in continuation of his work on orchids has published upward of 20 new species from tropical America. One new genus (*Ischnogyne*) from the mountains of Setschuan, China, is included.—C. W. SHANNON (Okl. Geol. Surv. no. 4. pp. 41. 1913) has issued a list of the trees and shrubs of Oklahoma.—J. K. SMALL (Torreya **13**:77. 1913) describes a new species of *Malpighia* (*M. Harrisii*) from Jamaica.—J. D. SMITH (Bot. Gaz. **55**:431–438. 1913) presents

his 36th paper, as a result of continued study on the flora of Central America. The article includes descriptions of 12 species of flowering plants new to science. —J. D. SMITH and J. N. ROSE (Contr. U.S. Nat. Herb. **16**:287–298. 1913) have published a "Monograph of the Hauyeae and Gongylocarpeae, tribes of Onagraceae." The study embraces 4 genera and 14 species; two new generic names are proposed, namely, *Xylonagra*, based on *Oenothera arborea* Kellogg, and *Burragea*, based on *Gaura fruticulosa* Benth.—W. W. SMITH (Rec. Bot. Surv. India **4**:324–431. 1913) records the results of a botanical survey of South-east Sikkim, India, lists 925 species, and describes a new genus (*Paroxygraphis*) of the Ranunculaceae.—C. SPEGAZZINI (Ann. Mus. Nac. Buenos Aires **23**:167–244. 1912) in an article on the Laboulbeniaceae of Argentina has published several new species and proposes two new genera, namely, *Cochliomyces* and *Laboulbeniella*. The same author (*ibid.* 1–146) under the title "Mycetes Argentinenses" continues the enumeration of the Mycetes of Argentina, adds several species new to science, and proposes the following new genera: *Eudimeriolum*, *Winteromyces*, *Trichospermella*, *Dasysphaeria*, *Criserosphaeria*, *Hormopeltis*, *Polhysterium*, *Symphaeophyma*, *Apiosporella*, *Ectosticta*, *Dasysticta*, *Dasyprena*, *Phaeopolynema*, and *Phaeolabrella*.—A. STEWARD (Proc. Calif. Acad. Sci. **1**:431–446. 1912) records the lichens found on the Expedition of the California Academy of Sciences to the Galapagos Islands in 1905–1906. Six-teen species were found which were not before reported from the islands.—H. and P. SYDOW (Ann. Mycol. **11**:93–118. 1913) have published several new species of fungi from northern Japan and characterize a new genus (*Miyagia*) of the Pucciniaceae found on leaves of *Anaphalis margaritacea*. The same authors (*ibid.* 254–271) under the title "Novae fungorum species X" have published several species new to science and propose the following new genera: *Aithaloderma*, *Schizochora*, *Cyclodothis*, and *Diedickea* from the Philippine Islands, *Astrosphaeriella* and *Coccidophthora* from Japan, and *Nematostigma* from South Africa.—C. TORREND (Broteria, Ser. Bot. **11**:73–98. 1913) under the title "Les Basidiomycetes des environs de Lisbonne et de la région de S. Fiel (Beira Baixa)" includes the descriptions of several new species and proposes one new genus, namely, *Lycoperdellon*, based on *Lycogala Torrendii* Bres.—I. URBAN (Bot. Jahrb. **50**: Beibl. 111. pp. 1–108. 1913) under the title "Plantae novae andinae imprimis Weberbauerianae VI" in cooperation with several specialists has published an important paper on the Andean flora. About 125 species new to science are described.—H. F. WERNHAM (Jour. Bot. **51**:218–221. 1913) has published 11 new species of Rubiaceae from tropical America. —R. S. WILLIAMS (Bryologist **16**:36–39. *pl. 4.* 1913) reports *Brachymenium macrocarpum* Card. from Florida and describes a new species of *Funaria* (*F. rubiginosa*) from Montana.—J. M. GREENMAN.

Metabolism of fungi.—Believing that methods based on a determination of the yield, or of the economic or the respiratory coefficients do not give a satis-factory quantitative representation of the manner of utilization of carbon

compounds by fungi, WATERMAN[2] has applied the conceptions of "plastic equivalent" and "respiratory equivalent" to the study of the carbon metabolism of *Aspergillus niger*. The "plastic equivalent" for carbon is defined as that fraction or percentage of the consumed carbon which at any given moment is contained in the substance of the organism. Similarly, the "respiratory equivalent" is the percentage which has up to that moment reappeared as carbon dioxide. Experimentally these relations are obtained by determinations of the carbon given off in respiration, the quantity fixed in the body of the fungus, and the total quantity that has been consumed.

The author has studied from this point of view the assimilation of glucose, levulose, mannose, and a number of organic acids. As a rule, however, only the plastic equivalents were determined, the respiratory equivalent having been determined only for succinic acid. The results show, apparently to the mild surprise of the author, that the plastic equivalent is high during the early stages of growth, and falls with increasing age of the culture; while the greater part of the carbon nutrient disappears during the first few days of growth. From these observations he arrives at his main conclusion, that the temporary accumulation of carbon in the fungus is due to the formation of an intermediate product which he finds to be glycogen. This view, of course, presents nothing novel, for it is well known that in the presence of an excess of food, reserve materials, which in fungi mostly take the form of glycogen, are stored in the plant body, and that respiratory activity, decreasing the "plastic equivalent," continues at the expense of reserve materials and even at the expense of proteids when the external food supply has been exhausted. All such substances must in this sense be regarded as intermediate products. In this connection it may be pointed out that from the discrepancy between the quantity of carbon dioxide developed by a fermenting mixture and that which should have been developed according to the quantity of glucose (determined by change in rotation) which disappeared from the mixture, EULER and JOHANSSON[3] have recently concluded that intermediate products were first formed from the glucose in the process of fermentation. Regarding the author's method it should also be stated that SPIEKERMANN,[4] without giving special names to the ratios, determined for a species of *Penicillium* growing on glycerin the percentage of the consumed carbon fixed in the plant body, and that given off in respiration.

Finally, WATERMANN has investigated the influence of various factors in relation to the plastic equivalent. Changes in temperature and in concentration of the nutrient medium do not influence the nature but only the rate of

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[2] WATERMAN, H. I., Beitrag zur Kenntnis der Kohlenstoffnahrung von *Aspergillus niger*. **Folia Microbiol. 1:422–485. 1913.**

[3] EULER, H., und JOHANSSON, D., Umwandlung des Zuckers und Bildung der Kohlensäure bei der alkolischen Gärung. Zeitschr. Physiol. Chem. **76**:347–354. 1912.

[4] SPIEKERMANN, A., Die Zersetzung der Fette durch höhere Pilze. Zeitschr. Unters. Nahrungs- u. Genussmittel **23**:305–331. 1912.

metabolism. The magnitude of the plastic equivalent is to a high degree dependent on the nature of the carbon nutrient. This relation is correlated with the heats of combustion of the carbon compounds. Those having the greater caloric value give the highest plastic ratios.—H. HASSELBRING.

Bud variations and fruit markings.—This very interesting question is the subject of a paper by KRAUS,[5] who has been making studies on the effects of cross-pollination of cultivated fruits. The author calls attention to the frequent occurrence of banded or striped fruits, especially among apples. The most common explanation of this phenomenon is the secondary influence of pollen, but the author explains that this cannot be true xenia, such as occurs in corn. Correspondence with horticulturists and botanists indicated a prevailing opinion that it is due to secondary influence of pollen, though a number believed it due to bud-variation. After explaining the economic importance of the problem, the author describes his methods of work. The conclusions are as follows: "color in the pome fruits is not influenced directly in the immediate cross; new characters cannot be added by the pollen, outside the seed itself, in the immediate cross; the manifestation of color is dependent on many environmental factors; color as usually found is composed of a number of unit characters; somatic segregation may occur and by this means the several factors of color manifest themselves more or less independently (the several colors may appear as bands more or less parallel, or a band of but one color surrounded by the normal color); similar segregation may extend to any group of unit characters of which the plant is composed; segregation may extend to either fruit or leaf buds; if the latter, such variations may be propagated asexually; red in apples may consist of either a single or a complex of unit characters; at least, three reds are recognizable; somatic segregation may be of service to plant breeders as indicating the unit characters of a plant that are likely to exhibit themselves when propagated sexually; segregation generally extends to the flower bud only in apples, while in pears the shoot is frequently affected."—MEL T. COOK.

The development of chalazogams.—NAWASCHIN and FINN[6] have published a contribution in German which extends the study of *Juglans* published in Russian a year ago and already noted in this journal.[7] The principal conclusions are: that in seed plants there is a tendency to reduce the male gametes from sperms to naked nuclei; that the evolution of the pollen tube and simplification of the sperm go hand in hand; that *Juglans* and other chalazogamous plants with a well developed binucleate cell which reaches the embryo

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[5] KRAUS, E. J., Bud variations in relation to fruit markings. Biennial Crop Pest and Horticultural Report for 1911 and 1912. Oregon Agric. Exp. Station. pp. 71–78.

[6] NAWASCHIN, S., and FINN, V., Zur Entwickelungsgeschichte der Chalazogamen. *Juglans regia* und *Juglans nigra*. Mém. Acad. Imp. Sci. St. Pétersbourg **31**:1–59. *pls. 1–4*. 1913.

[7] BOT. GAZ. **55**:94. 1913.

sac in fact show a condition intermediate between a well developed sperm and a naked sperm nucleus; and that this feature indicates the great age of chalazogams. These conclusions, which are practically the same as those given in the previous paper, are based upon a large amount of research and also upon a thorough discussion of the literature, in which the work of American investigators receives generous recognition. Both authors had already become identified with the subject, and no one has contributed more to our knowledge of chalazogamous plants than NAWASCHIN. Besides, as the discoverer of "double fertilization," he has made a reputation for brilliant initiative in research, while his more recent investigation of the sperm nucleus of *Lilium Martagon* entitles him to a place among the authorities in cytological matters. These facts lend weight to the conclusions. The paper deserves a careful reading by everyone who attempts to treat the phylogeny of angiosperms from a cytological standpoint. Three of the large plates are colored, and the fourth (copied from various investigators) gives a useful optical survey of pollen tube structures in various groups of gymnosperms and angiosperms.—CHARLES J. CHAMBERLAIN.

Cecidology.—Among the important foreign contributions is a purely botanical paper by BUYSSON and PIERRE,[8] in which two species occurring on *Sedum* are discussed. HOUARD[9] gives good descriptions of a number of cecidia in the Natural History Museum of Paris, restricting his discussions to the galls and not to the causes. KIEFFER[10] describes two new genera and two new species of cecidomyid galls and gall-makers from Formosa.

Among the most interesting American contributions is a very suggestive paper on seedless and malformed fruits by BROWN,[11] in which the author, after discussing malformations and russetings caused by frost, also calls attention to the fact that fruits may be abnormal as a result of no pollination or imperfect pollination combined with frost injuries. After pollination, a severe frost may interfere with the fertilization processes and affect both seed and fruit. The author also states that there is relationship between weights of seeds and fruit but does not give figures.

FELT[12] contributes a very valuable entomological study of gall midges, in which he includes keys, descriptions, and drawings of a great many species. —MEL T. COOK.

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[8] BUYSSON, H. DU, et PIERRE, l'ABBE, Nouvelles cecidologiques du centre de la France. Marcellia 12:27–35. 1913.

[9] HOUARD, C., Les collections cécidologiques du laboratoire d'entomolgie du. Museum d'histoire naturelle du Paris: Galles du Mavor. Marcellia 12:35–41. 1913.

[10] KIEFFER, J. J., Description de deux remarquables cécidomyies de Formose. Marcellia 12:42–44. 1913.

[11] BROWN, F. R., Seedless and malformed fruits. Biennial Crop Pests and Horticultural Report for 1911 and 1912. Oregon Agric. Exp. Station.

[12] FELT, E. P., A study of gall midges. Twenty-eighth Report of the Entomologist of the State of New York. pp. 127–226. 1913.

Parthenogenesis in Bennettites.—Parthenogenesis, or the development of an embryo from an egg without fertilization, has been demonstrated for several angiosperms, and is claimed for *Pinus pinaster* and *Gnetum Ule*. In spite of the difficulty of proving a case of parthenogenesis in living seed plants, LIGNIER[13] has given us a short paper with the rather startling title "*Bennettites Morieri* probably reproduces by parthenogenesis." One naturally looks for the evidence. Here it is. He found embryos, but no pollen grains or pollen tubes, and the tip of the nucellus was closed. Some of the sections were in series, at intervals of about 3 mm. Presumably most of the sections were not in series. When one remembers that pollen tubes have never been demonstrated in the Bennettitales and that very possibly the pollen grains may shed their sperms without the formation of a pollen tube, and that even in rather thick sections at intervals of 3 mm., more than nine-tenths of the material is missing, the evidence is not convincing. And yet, this "probable parthenogenesis" is given as a reason for the rapid disappearance of this group during the Cretaceous.—CHARLES J. CHAMBERLAIN.

A classification of conifers.—SAXTON[14] has proposed a classification of conifers based upon the great extension of knowledge of the group developed during recent years. There is no question that the current classifications are archaic, and that a more natural classification of the group is demanded. SAXTON analyzes the characters to be used in such a classification, and discusses the various attempts that have been made. He then describes, with definite characters, five families (Araucariaceae, Podocarpaceae, Pinaceae, Cupressaceae, and Taxaceae), Pinaceae having two subfamilies (Abietoideae and Sciadopitoideae) and Cupressaceae three (Cupressoideae, Callitroideae, and Sequoideae). A consideration of the phylogeny of Coniferales results in an interesting "family tree" that shows the relationships of the families and subfamilies. An interesting item of the phylogeny, at this time, is that the araucarians are represented as the first offshoot from the common stock (presumably abietinean) that arises from Cordaitales, which later gave rise to the podocarps, and then the other families.—J. M. C.

Artificial parthenogenesis in Fucus.—Parthenogenesis in various members of the Phaeophyceae has been known for some time, and unfertilized eggs of *Fucus* have been caused to divide by treating them with solutions. OVERTON[15] took exceptional care to obtain unfertilized eggs of *Fucus vesiculosus*, and each collection was divided into three lots, one of which was then fertilized, another was allowed to remain in normal sea water, while the third was treated with

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[13] LIGNIER O., Le *Bennettites Morieri* (Sap. et Mar.) Lignier se reprodusait probablement par parthénogénèse. Bull. Soc. Bot. France IV. **11**:125–127. 1911.

[14] SAXTON, W. T., The classification of conifers. New Phytol. **12**:242–262. 1913.

[15] OVERTON, J. B., Artificial parthenogenesis in *Fucus*. Science **37**:841, 844. 1913.

acetic or butyric acid. The general result of the various cultures was that in the first case the sporelings developed normally; in the second, no sporelings appeared; while in the third, sporelings developed up to the 25-cell stage. The experiments were not carried farther. No cytological work was done. Since the *Fucus* plant is the *2x* generation, it would be interesting to know the chromosome situation, especially if the plants should develop up to the reproductive phase.—CHARLES J. CHAMBERLAIN.

Chromosome conjugation.—Miss FRASER,[16] in a short discussion of chromosome conjugation, cites the work of OVERTON, HARPER, DIGBY, and others to show that it is not a matter of primary importance whether parasynapsis or telosynapsis takes place, and that they need not be mutually exclusive. The sexual nuclei may fuse at once upon fertilization, or not until the division of the oospore in other cases (Pinaceae); while in the extreme case of the rusts they remain distinct until just before meiosis. In like manner the attraction between the homologous chromosomes may bring about their conjugation as soon as the nuclei fuse, or in other cases later, even as late as the formation of the gemini of maturation. The suggestion is made that the clearest cases of Mendelian inheritance will perhaps be found to be those correlated with a late association of the chromosomes in pairs.—L. W. SHARP.

Flora of Boulder.—DANIELS[17] has made a study of the vascular flora of Boulder, Colo., and vicinity, a most interesting mountain region. An introduction (48 pp.) describes the physiography, the climate and rainfall, and the zones of vegetation. The zones given are Campestres, Mensales, Submontanae, Montanae, Subalpestres, and Alpestres, each with numerous subdivisions. The list of plants (211 pp.) includes 1225 numbers in 486 genera, with a statement as to the habitat of each species. A number of new combinations are made, and new species described in *Acomastylis* (*Geum*), *Prunus*, *Vitis*, *Castilleja*, and *Grindelia*. One of the unique features of the list is that a popular name is given for each species. When this reaches such a stage as "filiform toad-flax-leaved painted cup," it is probable that it ceases to be useful.—J. M. C.

Graft hybrids.—Miss HUME[18] has investigated three graft hybrids for connecting protoplasmic threads. The "periclinal chimaeras" used were *Cytisus Adami*, *Solanum tubingense*, and *S. Kolereuterianum*, since in these the epidermis is the only layer of cells belonging to the one component, and the line of demarcation between the two components is therefore a sharp one. BUDER

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[16] FRASER, H. C. I., The pairing of the chromosomes. New Phytol. **11**:58–60. 1912.

[17] DANIELS, FRANCIS POTTER, The flora of Boulder, Colorado, and vicinity. Univ. Missouri Studies. Science Series **2**: no. 2. pp. xiii+311. 1911.

[18] HUME, MARGARET, On the presence of connecting threads in graft hybrids. New Phytol. **12**:216–221. 1913.

had already discovered connecting threads between the two components in *Cytisus Adami*, which Miss HUME confirmed. In *Solanum tubingense* she found them, while in the other *Solanum* she did not. These results show that genetically unrelated tissues can be joined by connecting threads, and the inference is that such threads do not arise from spindle fibers, since no nuclei of the two components have even been sisters.—J. M. C.

The embryo sac of Bellis.—The peculiar development of the antipodal region in some Compositae has long been known. In 1895 the reviewer[19] described an "antipodal oosphere" in *Aster novae-angliae*, and nearly ten years later Miss OPPERMAN[20] not only found an antipodal oosphere in *Aster undulatus*, but noted its fertilization. Recently, CARANO[21] has described a "pseudo-oosphere" in the antipodal region of *Bellis perennis*. In all these cases, the region in which the abnormal oosphere is found is greatly enlarged; in fact, it has the appearance of an embryo sac. Doubtless the conditions in the enlarged antipodal cell are about the same as in a normal embryo sac, and consequently the occasional organization of an oosphere is not so strange as we formerly supposed.—CHARLES J. CHAMBERLAIN.

Reproduction in gymnosperms and angiosperms.—Under this title ERNST[22] gives an excellent résumé of the present status of the subject. The illustrations, which are taken from the leading contributions, are well reproduced. The bibliographies are in two categories: those which treat the subject in a general way, like textbooks, and those which deal with original investigation. The title, *Hand dictionary of the sciences*, is somewhat misleading for English-speaking people, for this "dictionary" is more like an encyclopedia, consisting of several volumes, edited by a staff of specialists. OLTMANNS is the general editor of botany, and he has distributed the various topics to specialists in the various fields. All articles, like the one just mentioned, are signed.—CHARLES J. CHAMBERLAIN.

Mitosis in Oenothera.—In the somatic divisions of *Oenothera lata* GATES[23] finds the chromosomes forming from the delicate reticulum by the parallel fusion of several strands. No prochromosomes are present, and no continuous spirem is formed. The splitting of the chromosomes occurs in late prophase,

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[19] CHAMBERLAIN, CHARLES J., The embryo sac of *Aster novae-angliae*. BOT. GAZ. **20**:205–212. *pls. 15, 16.* 1895.

[20] OPPERMAN, MARIE, A contribution to the life history of *Aster*. BOT. GAZ. **37**:353–362. *pls. 14, 15.* 1904.

[21] CARANO, ENRICO, Su particolari anomalie del sacco embrionale di *Bellis perennis*. Annali di Botanica **11**:435–439. *pl. 9.* 1913.

[22] ERNST, A., Fortpflanzung der Gymnospermen und Angiospermen. Abdruck aus Handwörterbuch der Naturwissenschaften **4**:227–261. *figs. 37.* 1913.

[23] GATES, R. R., Somatic mitosis in *Oenothera*. Ann. Botany **26**:993–1010. *pl. 86.* 1912.

but the split disappears temporarily before the metaphase, when it is again evident. During late prophase and metaphase the chromosomes often show a distinctly paired arrangement. In telophase there is a massing at each pole, after which the chromosomes separate and become joined by anastomoses. Considerable variation in chromosome number is shown; the usual number is 15. The nuclei occasionally show certain characters of heterotypic mitosis.—L. W. SHARP.

Poison weed.—Larkspurs have always had the reputation of being poisonous, but it seems that only in North America have they been important in causing losses of stock. MARSH and his associates[24] have investigated the poisoning due to larkspur in Colorado, and presumably the conditions are the same in other mountain cattle ranges of the West. The larkspurs are grouped as tall and low larkspurs, *Delphinium Barbeyi* representing the first group, and *D. Nelsonii* the latter. These forms cause the loss of a great number of cattle, but horses and sheep are not injured by grazing on larkspur areas; and cattle are not injured if prevented from grazing on such areas until the second week of July. The next problem is to discover the specific poison.—J. M. C.

Xenia.—Having discovered instances of xenia in wheat, BLARINGHEM[25] sought to determine whether the vigorous development of hybrid enbryo and endosperm ever causes a change in the character of the maternal tissue that surrounds them. On crossing a comparatively small wheat, known as *Triticum turgidum gentile* Al. var. Normandy, with a larger type, *Triticum vulgare lutescens* hybrid no. 126 of the Hohenheim collection, 16 hybrid seeds approaching the paternal variety in size were obtained. This phenomenon is interpreted as xenia in the original sense of the term, though it seems probable that it is simply a stretching of the pericarp due to a large hybrid embryo and endosperm.—E. M. EAST.

Artificial cell structure.—In a series of interesting experiments, W. MAGNUS[26] has produced, from paraffin, beeswax, and other substances, various structures which bear a striking resemblance to cells and tissues. The paraffin, with a melting point of about 74° C., was poured upon mercury which had been heated to 78° C. and allowed to cool at room temperature. While this is only the beginning of the investigation, the writer thinks he has already shown that through the action of purely physical forces structures can be produced which look like

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[24] MARSH, C. DWIGHT, CLAWSON, A. B., and MARSH, HADLEIGH, Larkspur or "poison weed." U.S. Dept. Agric., Bur.,Plant Ind., Farmer's Bull. 531. 1913.

[25] BLARINGHEM, L., L'influence du pollen visible sur l'organisme maternal; découverte de la xénie chez le blé. Bull. Soc. Bot. France **60**: 187–193. 1913.

[26] MAGNUS, WERNER, Über zellenformige Selbstdifferenzierung aus flüssiger Materie. Ber. Deutsch. Bot. Gesells. **31**: 290–303. *pl. 13*. 1913.

cells. Further work is expected to show to what extent the physical forces concerned in the living and inorganic material are identical.—CHARLES J. CHAMBERLAIN.

Ovulate flower of Gnetum.—The publication of Miss BERRIDGE's paper[27] on the ovulate strobilus of *Gnetum Gnemon*, in which she gave evidence for the conclusion that the ovule was "primitively surrounded by a whorl of male flowers," has called out a paper by LIGNIER and TISON[28] upon the same structure. They have found material that seems to indicate that the "third integument" of the ovule is a modified axis of inflorescence that bore an axillary ovule; and that occasionally an axillary group of staminate flowers is present, which indicates a connection with *Welwitschia*.—J. M. C.

Beech forest on chalk and on schist.—Comparing the English beech forests on chalk with the French on schist SKENE[29] finds that they are exactly similar ecologically, and that scarcely a member of the latter is a calcifugous plant, while scarcely a single member of the former is a calcicolous plant. Topographically there is no distinction between the two types. This leads SKENE to question the accuracy of placing the two forests in different formations according to the classification adopted by British ecologists.—GEO. D. FULLER.

A bibliography of mitosis.—A very useful list of works on meiosis and somatic mitosis in the angiosperms since 1880 has been compiled by PICARD.[30] The forms are arranged according to systematic position. Although the author has not attempted to make the citations on the individual plants exhaustive, the 300 and more citations given justify him in his belief that from the list one can obtain reference to all the literature of the subject.—L. W. SHARP.

Embryogeny of the Ranunculaceae.—In continuing his studies of the Ranunculaceae, SOUÈGES[31] has described the development of the embryo of *Ficaria ranunculoides*, including some interesting cytological details.—J. M. C.

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[27] See BOT. GAZ. **55**:172. 1913.

[28] LIGNIER, O., et TISON, A., L'ovule tritégumenté des *Gnetum* est probablement un axe d'inflorescence. Bull. Soc. Bot. France **60**:64–72. *figs. 5*. 1913.

[29] SKENE, MACGREGOR, The relation of the beech forest to edaphic factors. Jour. Ecol. **1**:94–96. 1913.

[30] PICARD, M., A bibliography of works on meiosis and somatic mitosis in the angiosperms. Bull. Torr. Bot. Club **40**:575–590. 1913.

[31] SOUÈGES, R., Recherches sur l'embryogénie des Renonculacées. Bull. Soc. Bot. France **60**:150–157. *pl. 11. figs. 288–315*. 1913.

Volume LVII Number 3

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

MARCH 1914

The Anatomy of Ophioglossum pendulum — Loren C. Petry

Some Effects of Colloidal Metals on Spirogyra — W. D. Hoyt

The Function of Manganese in Plants — W. P. Kelley

The Development of the Prothallium of Camptosorus rhizophyllus — F. L. Pickett

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THE BOTANICAL GAZETTE

MARCH 1914

THE ANATOMY OF OPHIOGLOSSUM PENDULUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 183

LOREN C. PETRY

(WITH SIXTEEN FIGURES)

The section OPHIODERMA of the genus *Ophioglossum*, as first separated by PRANTL (**6**), is distinguished from EUOPHIOGLOSSUM by the numerous vascular strands in the base of the petiole, and from CHEIROGLOSSA, which shares this feature, by the occurrence of a single spike on each fertile leaf. As originally described, the section was composed of the single species *O. pendulum* L.; *O. intermedium* Hook. was considered to be a young form of *O. pendulum*, and was included with that species. In 1904, BOWER (**2**) described the new species *O. simplex* Ridley, from Sumatra, and from an examination of its vascular system showed that it is a member of this section. He further reestablished the species *O. intermedium* Hook. on the basis of its intermediate position between *O. pendulum* and *O. simplex*.

Of the three species constituting the section, *O. pendulum* is best known. BOWER (**1**) has described the development of the fertile spike and sporangia, and in a later paper (**2**) has given a brief account of its anatomy. This paper deals merely with the vascular system of the leaf and its insertion upon that of the stem. The investigation here described has been undertaken with a view to supplementing this account of the anatomy of the species.

Material

A large number of entire plants was collected by Dr. CHARLES J. CHAMBERLAIN in November 1911, in Northern Australia. The exact locality is near Babinda, in the Cairns district of Queensland, at a distance of about 25 miles from the coast. Occasional clusters of plants were found near the ground, but more usually they occurred at a considerable height, up to 30 meters. In almost all cases they were growing in the masses of humus accumulated by large plants of *Polypodium rigidulum; Lycopodium phlegmaria* was a common associate. Plants of this species were also observed on Stradbroke Island, near Brisbane, but no collections were made. The groups of plants here were at a less distance from the ground; they were commonly attached to plants of *Platycerium alcicorne* and *P. grande*.

In the material secured at Babinda, the largest leaves had attained a length of about 1.5 meters. However, some of the plants on Stradbroke Island had leaves nearly 2 meters long, and Mr. WM. GIBSON, a collector familiar with that region, reported a specimen with leaves measuring 2.7 meters in length. The largest spikes seen measured 30 cm. in length and 1.2 cm. in greatest width. Many cases of branching or lobing of the spikes were observed; probably one-tenth of the specimens showed some variation from the simple form.

This supply of entire plants was supplemented by a large number of rhizomes, root tips, buds, and spikes collected by Dr. W. J. G. LAND on the island of Tutuila, Samoa, in October 1912. These plants, together with many ferns and occasional plants of *Lycopodium phlegmaria*, occurred in the root masses of *Asplenium nidus*, at a height of 2–10 meters; a large cluster is shown in fig. 1. The leaves reached a length of 2 meters; branching of the leaf was not uncommon, but branching or lobing of the spike was relatively rare. The largest spike collected measured 51 cm. in length and 1.3 cm. in width; sporangia occurred along 45 cm. of its length. Several spikes more than 40 cm. in length were found.

The Australian material was preserved in 6 per cent formalin; that secured in Samoa was killed in 50 per cent alcohol and 6 per cent formalin. Even the largest rhizomes cut readily in paraffin

at 10–25 μ, and complete series were easily obtained. The safranin-anilin blue stain combination gave the best results.

FIG. 1.—*Ophioglossum pendulum*, epiphytic upon *Citrus* sp.; Tutuila, Samoa; photograph by Dr. W. J. G. LAND.

The root

The root stele at the point of attachment to the stem cylinder varies from diarch to pentarch; triarch and tetarch arrangements of the xylem are commonest. In passing from the base of the root toward the tip, the number of protoxylem points frequently increases by the splitting of one of the original protoxylem strands; in one case, the disappearance of a protoxylem strand was noted. In a few cases only, a hexarch arrangement was found; these roots were all very large. In general there is a definite relation between the size of the root and the number of protoxylem strands.

Each phloem mass is a layer 1 or 2 cells in thickness, separated from the xylem by 3–5 layers of parenchyma. It is easily distinguished by the somewhat thickened walls and the conspicuous "proteid granules" adhering to the walls. In the apical region of the root, the protophloem can usually be distinguished considerably in advance of the protoxylem. When a protoxylem strand splits, the phloem between the branches of the split strand connects with the phloem masses on either side.

There is no pericyclic layer; the phloem abuts directly upon the endodermis (fig. 10), which can be readily distinguished by the suberized band upon the radial walls; it resembles the endodermis of *Botrychium virginianum* in all particulars. Two or three cells of parenchyma separate the protoxylem from the endodermis.

Two general regions of the cortex about equal in thickness are distinguishable. In the inner region, the cell walls are heavily thickened by cellulose; simple circular or oval pits occur. In the outer region, the thickening of the cell walls is much less and gives the reactions of pectin compounds. Intercellular spaces of considerable size occur in the inner region; in the outer region, these are much smaller and become filled by the substance which constitutes the thickening of the cell walls. The endophytic fungus which occurs commonly in the roots is confined to the outer region.

The outer walls of the surface cells are remarkably thickened, as shown in fig. 2. The thickening material is not cellulose, but gives the reaction of pectin compounds. The thickening may continue until the lumen of the cell is almost filled; even in these con-

ditions the protoplasm remains very active. When cells of the epidermal layer are killed, the outer walls of the cells immediately below begin to thicken in this way. This may occur in any of the cells of the outer region of the cortex upon destruction of the overlying cells.

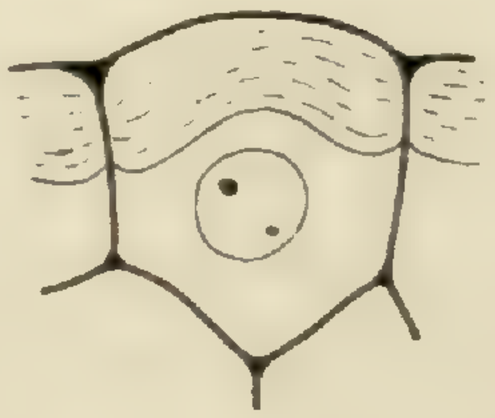

FIG. 2.—Epidermal cell of root; ×236.

Roots originate in the meristematic region of the stem by the formation of an apical cell in the first layer of cells outside the phloem. Fig. 3 represents this origin as seen in a longitudinal section of the stem. As shown, differentiation of the phloem has proceeded to within a few cells of the position of the root meristem. The growth of the root proceeds by the segmentation of the apical cell, which is tetrahedral in form; in slowly growing roots the segmentation is irregular, and a definite root cap cannot be distinguished. The first xylem elements are smaller in cross-section than those formed later; they are true tracheids with walls reticulately thickened. In a few cases of more rapidly growing roots, the thickening of the walls approaches the annular marking. This character of the protoxylem indicates the slow growth of the roots. The lignification proceeds slowly toward the center of the stele, and xylem parenchyma is often found in the center of the stele of large roots at a distance of 3–4 cm. from the tips. There is no secondary thickening of the xylem mass. In a few cases a medullated

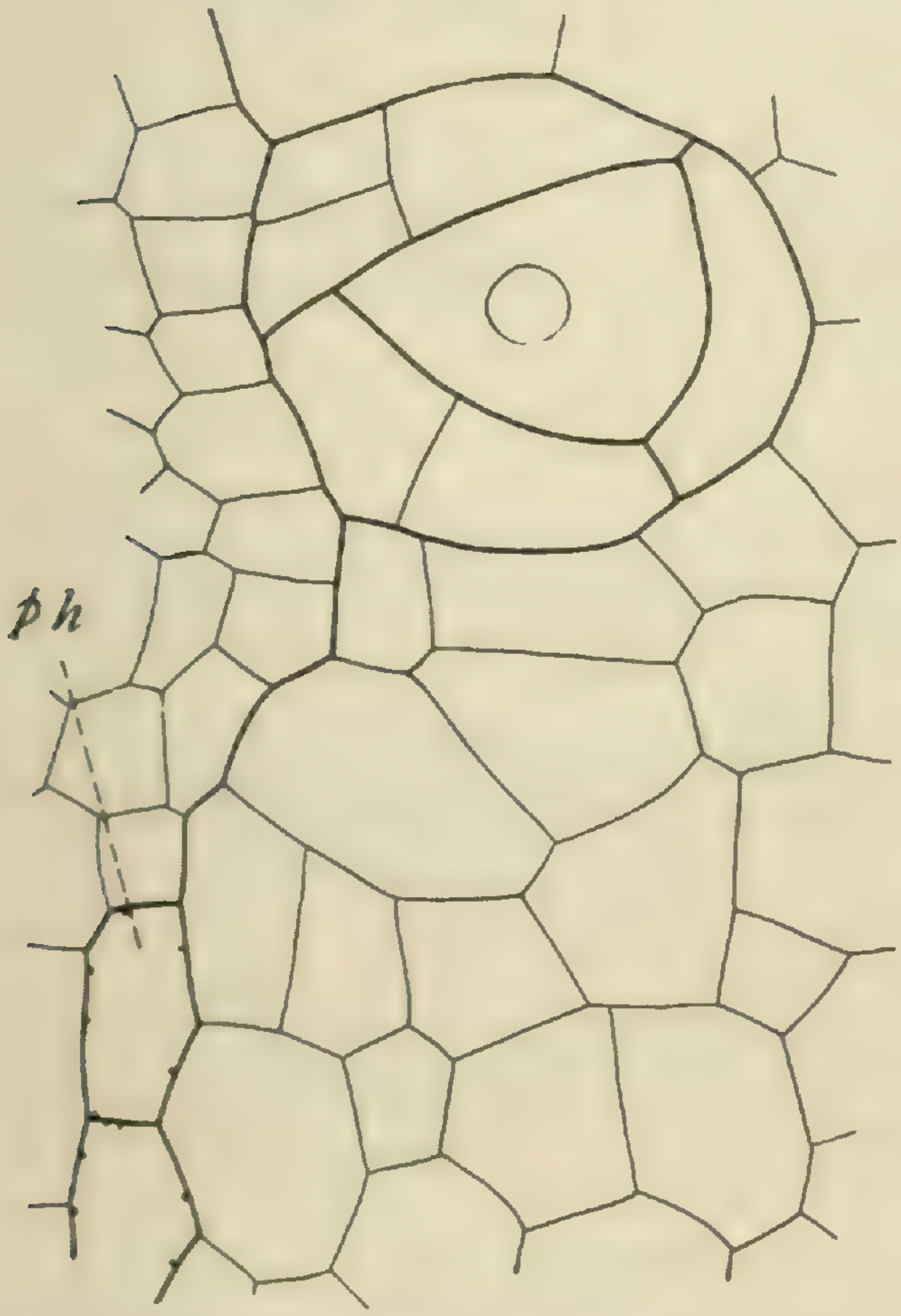

FIG. 3.—Origin of root, as shown in longitudinal section of a bud: *ph*, phloem; ×236.

condition occurred, as shown in fig. 4; the pith is not xylem parenchyma, but is composed of cells resembling those of the cortex; the opening through the xylem always occurs, but the endodermis is not interrupted. This occurs only in roots upon which buds have developed stems; the open portion of the xylem mass is upon the upper side of the root. This condition persists for only a short distance from the bud toward the tip of the root; the gap soon closes and a solid xylem mass is again formed. This peculiar root stele form is directly related to the formation of the stem stele from buds upon the roots, as described later.

The branching of the root is strictly monopodial. The lateral roots grow at an angle of 60–90° to the main root; two or three branch roots often develop at one point. These are not restricted in their growth, as in *Helminthostachys*, but may reach as great length as the primary roots.

FIG. 4.—Medullated root stele: *x*, xylem; *ph*, phloem; *e*, endodermis; ×37.

Immediately before a branch is given off, the phloem of the main stele spreads laterally between the endodermis and the protoxylem, and forms a continuous sheath about the xylem. A strand of xylem from each of two poles of the main root runs into the branch; hence the lateral roots at the point of attachment are diarch. The phloem sheath surrounds the xylem of the branch in the same way that it does that of the main root; the endodermis also connects without a gap. After the branch has separated, the phloem between the protoxylem and the endodermis disappears, and the usual arrangement of the stelar elements is restored.

In all cases, the branch stele is diarch at the point of attachment, but it may arise from a single pole of the main root. The protoxylem strands of the branch soon split, and the usual triarch or tetrarch condition is established. In one case, a branch arises from a large tetrarch root in which lignification has just begun; each pole is represented by three or four tracheids only. Two distinct strands of xylem, connecting with two poles of the main root, enter the branch. Each xylem strand is surrounded by a sheath

of phloem and endodermis. At a little distance, the endodermal sheaths come into contact, then fuse to form a single sheath; the phloem shows the same behavior. Xylem parenchyma then appears between the two protoxylem strands and the usual diarch condition of the branch stele is established.

The bud

The vegetative reproduction of the species is accomplished by buds upon the roots, as in *O. vulgatum*. As many as three buds were observed upon a single root; the second leaf of the oldest bud was just appearing, while the youngest bud was a mere swelling about 5 mm. from the root tip. It is almost certain that the plants of a colony have all developed from a single plant by this method of vegetative propagation; every rhizome examined in which the base is intact shows by its connection with a root that it has developed from a bud; fig. 5 represents such a case.

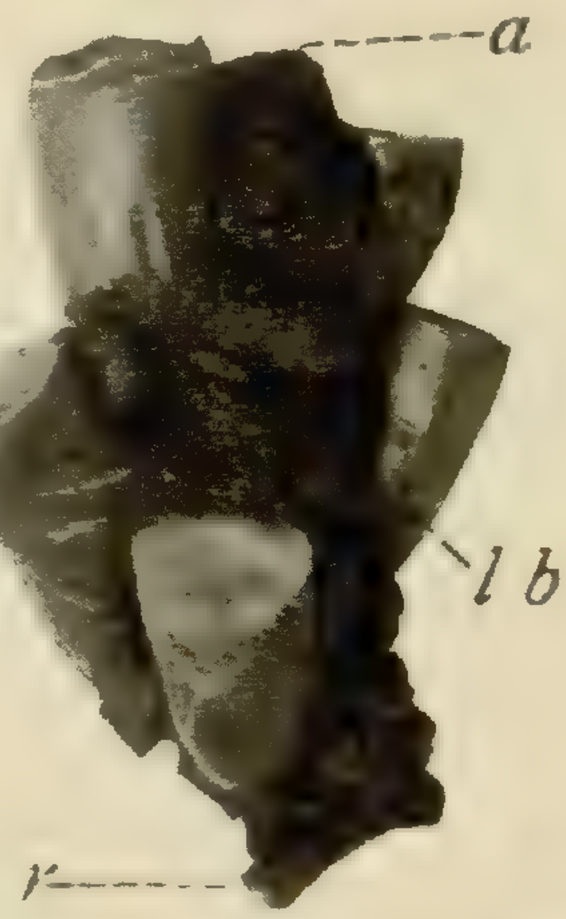

FIG. 5.—A rhizome showing radial character: *a*, stem tip; *lb*, base of a decayed leaf; *r*, parent root; ×1.

ROSTOWZEW (7) has described the development of the bud in *O. vulgatum*. In the second segment of the apical cell of the root a new apical cell arises; this produces all stem tissues. The root apical cell is retarded for a time, but finally resumes growth. This results in the formation of a bud with its axis approximately at right angles to the parent root.

The development of the bud has been examined in *O. pendulum;* it agrees in all important points with that of *O. vulgatum*. The retardation of the root growth is usually less; the bud axis often diverges less abruptly from that of the root. Two roots usually develop upon a bud before the first leaf is formed. The details of the vascular connection between the stem developed from a bud and the parent root will be described later.

The stem

The largest rhizome of the Australian material measures 0.7 cm. in diameter and 2.5 cm. in length. There are 18 leaves still attached and the bases of 5 others are evident. All the rhizomes, with the exception of the very youngest, give evidence of having grown in a horizontal position; field observations by Dr. CHAMBERLAIN confirm this. The leaf bases, however, are not restricted to the dorsal side, as stated by CAMPBELL (3) for this species, but are attached in a spiral about the stem. The distribution is irregular; on young stems the leaves are rather crowded and attached along a spiral with about a $\frac{1}{3}$ arrangement. On older stems the distribution approximates a $\frac{3}{5}$ arrangement; fig. 6 shows the arrangement of the leaves on the largest specimen secured. The bases of the leaves inserted on the lower side curve round the rhizome and produce the appearance of a 2-ranked arrangement, as in *Helminthostachys;* but there is nothing in the insertion of the leaves or the structure of the stem to indicate true dorsiventrality.

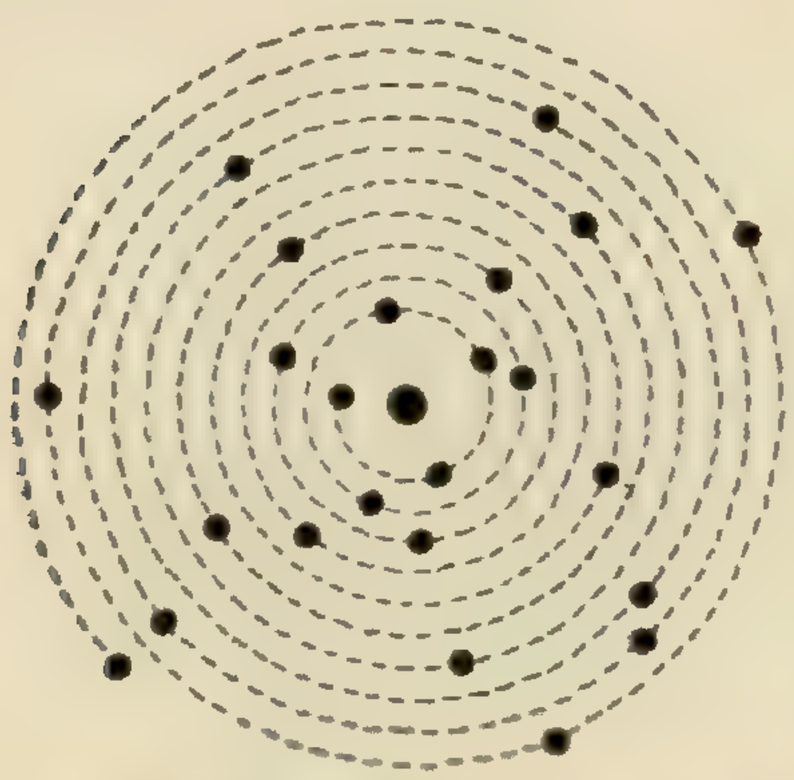

FIG. 6.—Diagram of leaf arrangement of a horizontally growing rhizome.

The rhizomes of the Samoan material are decidedly larger than those from Australia; the oldest one secured, with 8 functional leaves and 7 leaf bases, was 1.2 cm. in diameter and 4.6 cm. in length. Both their appearance and their structure indicate a definite radial arrangement (fig. 5). The leaf insertion is similar to that of the Australian specimens, but the leaves are less crowded.

All the rhizomes in which the bases are intact give evidence of having developed from buds upon roots in the manner described above. The connection of the stem stele with that of the root was examined in 12 specimens; five methods of development of the stem stele were found.

In a single specimen, a solid strand of xylem, surrounded by phloem and endodermis, separates at a considerable angle from one of the protoxylem strands of a tetrarch root (fig. 7). The endo-

dermis soon disappears; before separation from the root stele is complete, a few cells of parenchyma appear near the center of the xylem mass (fig. 7, *C*). The number of these increases and a definite pith is established (fig. 7, *D*). A small gap opens through the xylem, and closes again almost at once; the phloem is not interrupted. The xylem cylinder dilates rapidly and becomes oval in section; root steles attach at the points of the oval, leaving large "root gaps" in the cylinder (fig. 7, *L*). A third root attaches between the two gaps (fig. 7, *M*), producing a small gap; the three gaps close at about the same level. The first strand of the trace

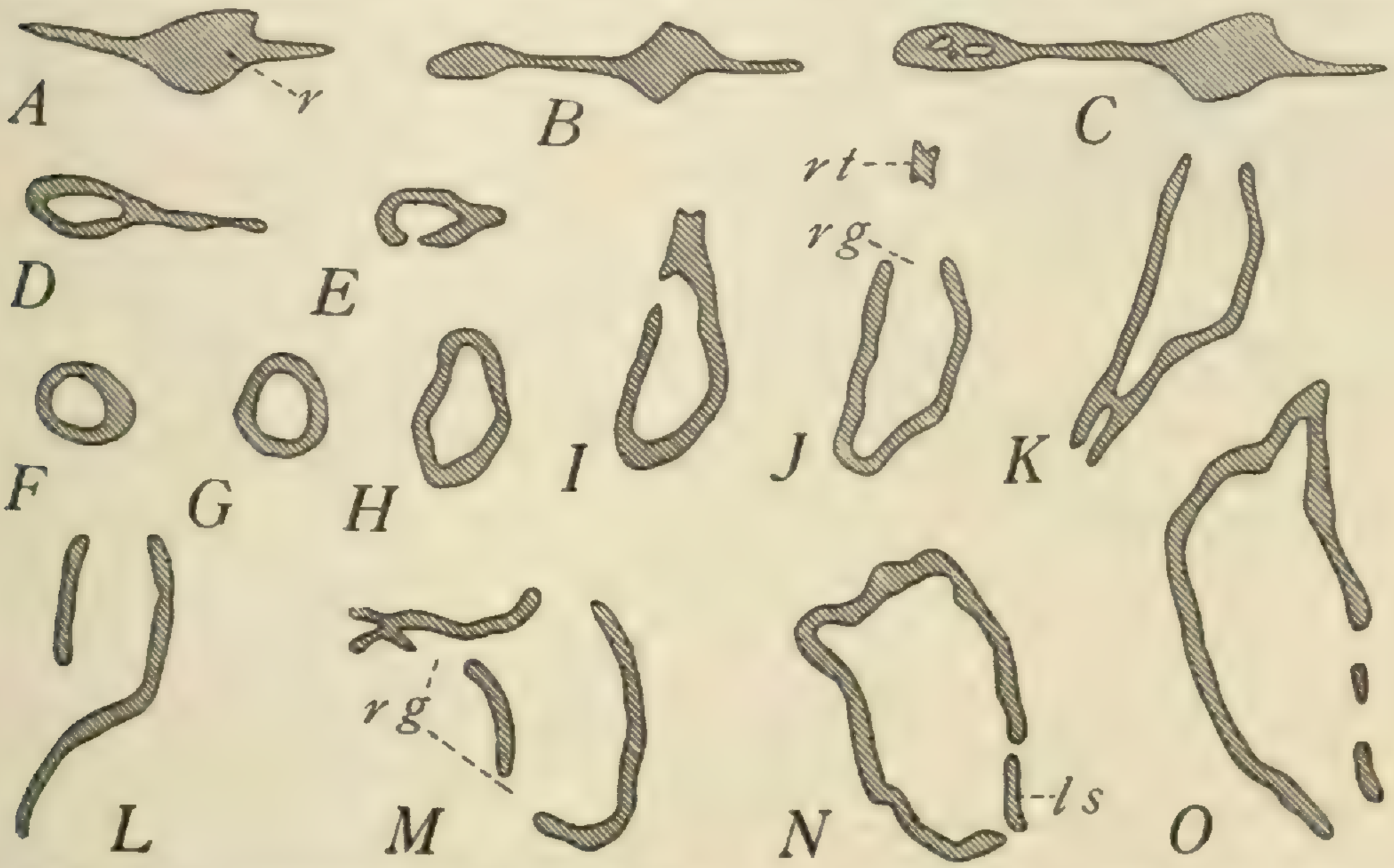

FIG. 7.—Development of stem stele from a single strand; only the xylem is shown: *r*, tetrarch stele of parent root; *rt*, root trace; *rg*, root gap; *ls*, first strand of first leaf; ×12.

of the first leaf separates from the cylinder at a slightly higher level (fig. 7, *N*). This is essentially the course of development of the stem stele of *O. vulgatum* from a bud.

In five cases, two strands of xylem of varying shape in cross-section separate from two of the poles of a triarch or tetrarch root. Diagrams of sections of one of these specimens are given in fig. 8. Each strand at the point of junction with the root stele has phloem on two faces, or surrounding it; the phloem disappears almost immediately from the adjacent faces and becomes restricted to the exterior sides of the strands. After an interval the two strands

fuse at one margin, forming a half-cylinder of xylem (fig. 8, *E*). Two roots are usually given off somewhat farther up (fig. 8, *I*), and the margins of the gap come together at about the level of the base of the first leaf.

In a single instance, three strands arise from the three poles of a triarch root. These fuse just below the point of connection of the first root to form a large strand of semicircular cross-section. The gap between the margins closes just below the level of the first leaf. In another specimen, two strands separate from each of two of the

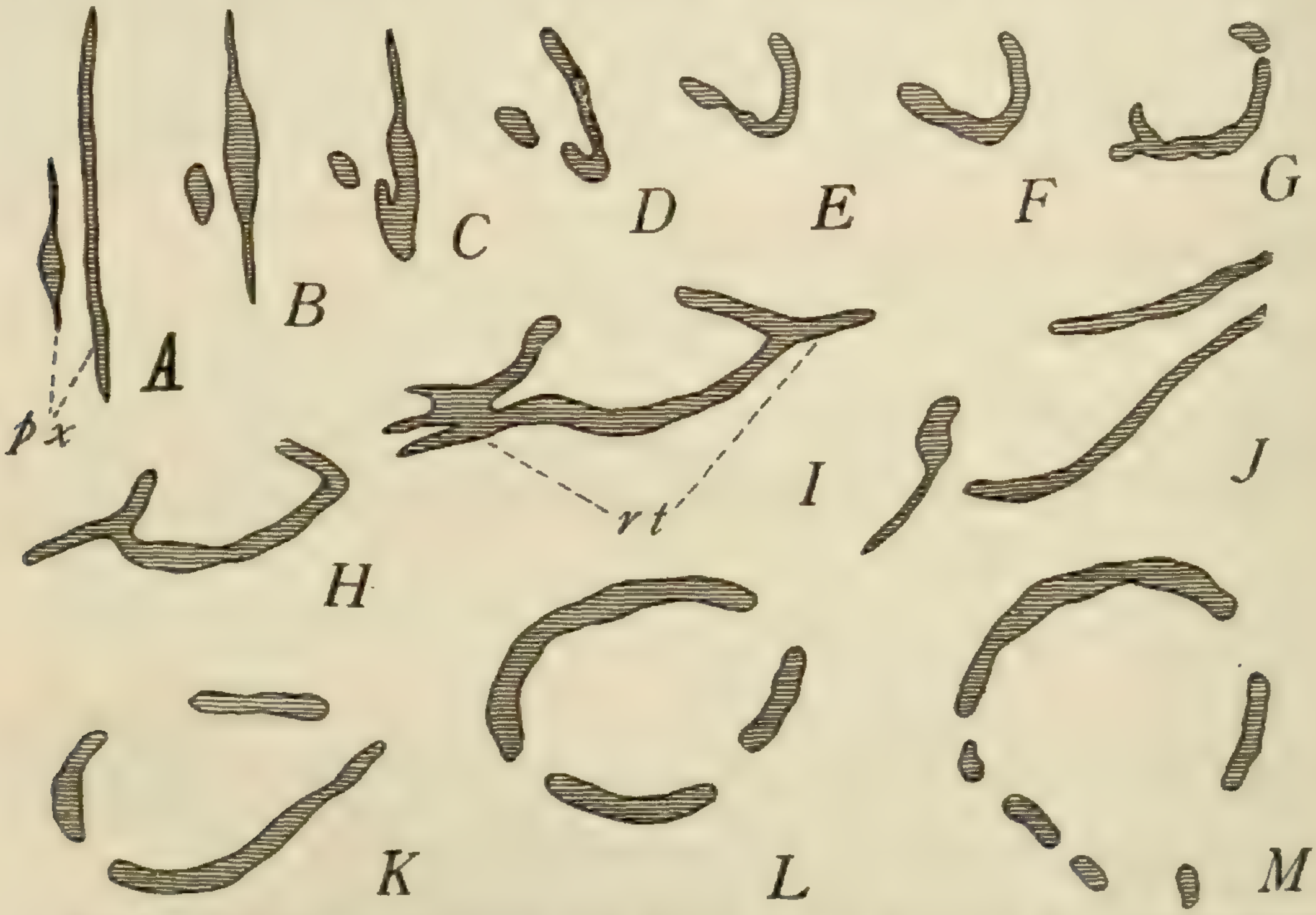

FIG. 8.—Development of stem stele from two strands; only the xylem is shown: *px*, protoxylem strands of parent root; *rt*, root traces; ×12.

protoxylem strands of a tetrarch root. These four organize a stele in the manner described above; the opening in the side is closed rather late, at the level of the top of the first leaf gap.

The stem stele is organized in a distinctly different way in four cases. A series of sections through one of the specimens from below upward is shown by fig. 9. In this case, the xylem mass of one pole of a diarch root begins to enlarge (fig. 9, *A*–*C*). After a time, medullation of the enlarged xylem strand occurs by the appearance of parenchyma near the center (fig. 9, *D*, *E*, fig. 10). The endodermis disappears on the side away from the unchanged

pole of the root. At the same level, gaps appear in the sides of the cylinder (fig. 9, *G*, *H*,) and the xylem separates into two strands. The smaller of these (*r*, fig. 9, *H*) is the continuation of the original root, and at once resumes its original diarch character. The larger strand organizes itself into the stem stele. The three strands of the first leaf separate (fig. 9, *I*, *J*), and at a slightly higher level (fig. 9, *J*, *K*) two roots are attached. The stem stele at this point

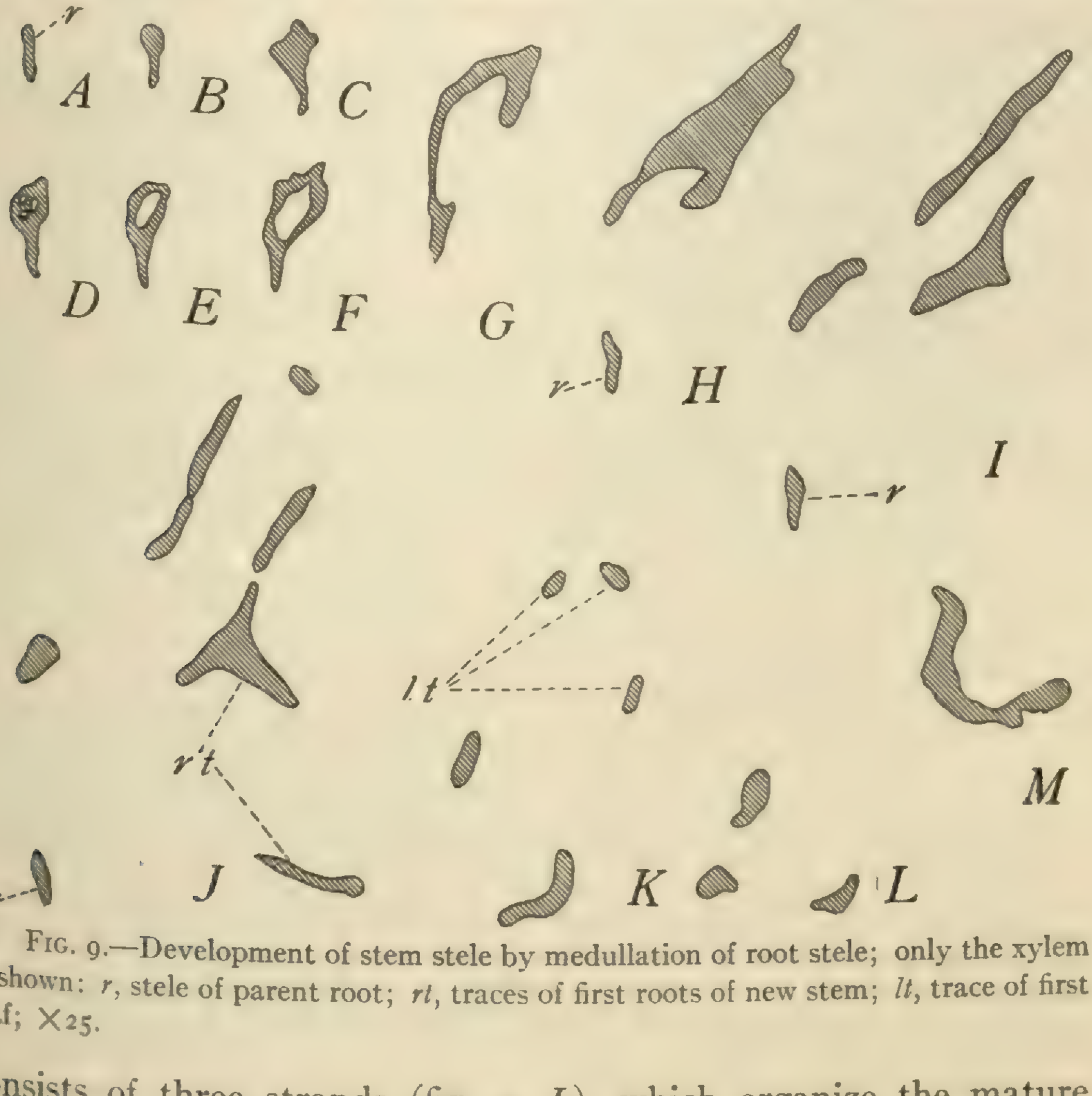

FIG. 9.—Development of stem stele by medullation of root stele; only the xylem is shown: *r*, stele of parent root; *rt*, traces of first roots of new stem; *lt*, trace of first leaf; ×25.

consists of three strands (fig. 9, *L*), which organize the mature form of the stele in the usual way. In the other rhizomes showing this manner of development, the original root is triarch or tetrarch; after separation from the stem stele, the root stele usually shows an increased number of protoxylem strands and the medullated condition shown in fig. 4.

In all cases, the stem stele soon assumes its usual form, that of an ectophloic siphonostele with more or less overlapping leaf gaps

(fig. 11). Two gaps usually occur in the same transverse section, and cause the xylem of the stem to appear as two curved masses with concave sides facing (fig. 11, *G*). Often only a single gap interrupts the cylinder (fig. 11, *A*), or the section may show a complete ring of xylem (fig. 12, *C*); very rarely, three gaps overlap. The leaf gaps are circular or oval and usually very large; 2 mm. in width by 2.5 mm. in height is an average size. The largest observed measures 3.2×3.5 mm.

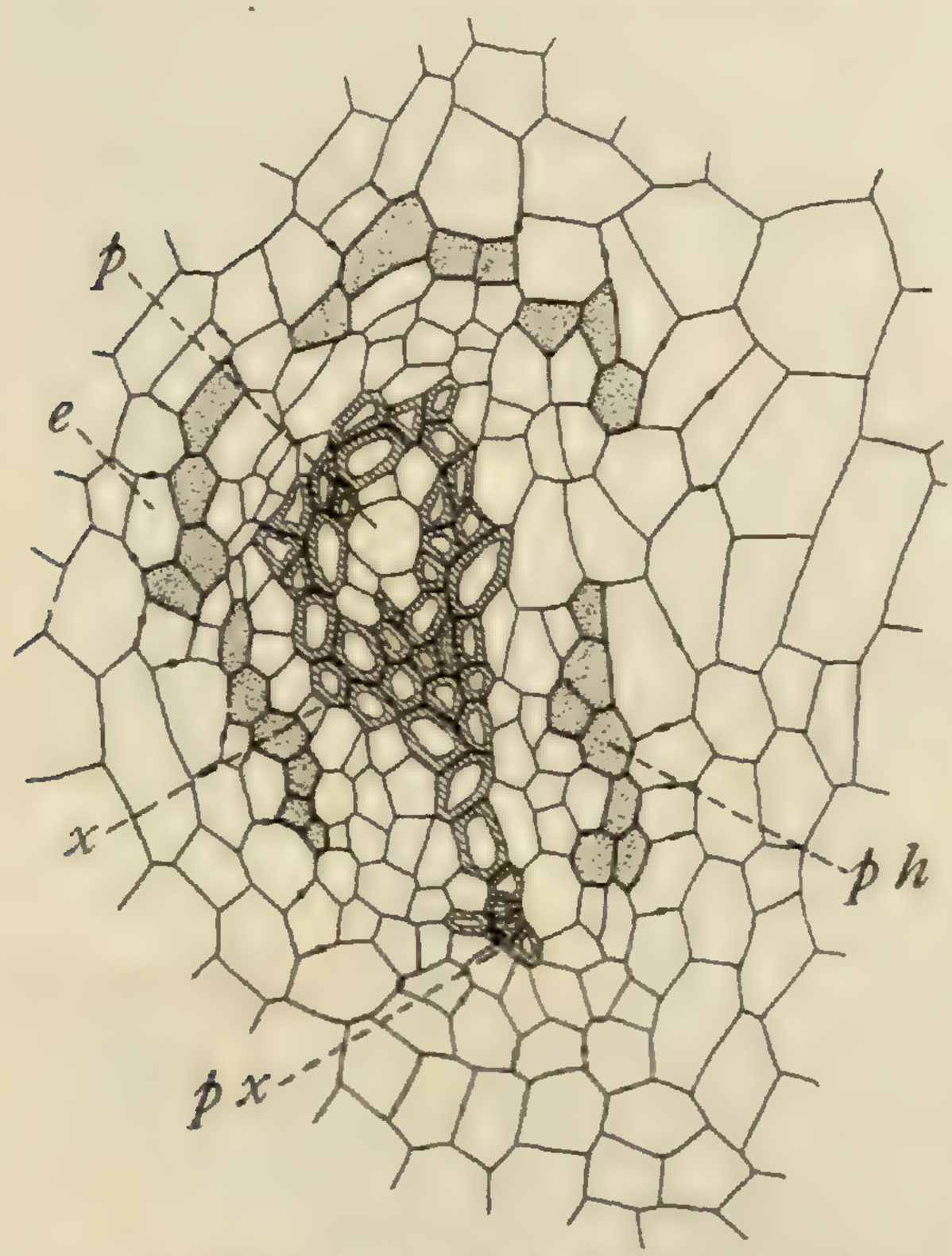

FIG. 10.—Detail of *E*, fig. 9: *x*, xylem; *ph*, phloem; *px*, protoxylem; *p*, parenchyma; *e*, endodermis; ×88.

In general the stele is more compact and shows more definitely its cylindrical character than in *O. vulgatum*. It is often very irregular in outline; the insertion of leaf and root strands usually produces a modification of shape, as shown in figs. 11, *I*; 12, *E*; 7, *J*, *K*, etc. It is usually not straight, but slightly bent at each leaf gap in the direction away from the leaf; this is undoubtedly due to the pressure of the young leaf. In diameter the stele is usually a little less than half that of the rhizome, that is, 3–5 mm.

Besides these leaf gaps, openings definitely related to roots occur in the cylinder (figs. 7, *J*, *L*; 8, *J*, *K*; 11, *B*, *C*, *J*). These are more conspicuous in the larger rhizomes, where a gap occurs above each root; in the smaller specimens, gaps occur in connection with about half of the roots. These openings are narrower in proportion to their length than are the leaf gaps; they range in size from 0.2×0.4 mm. to 0.5×2 mm. Where a root is attached to the cylinder immediately below the point of insertion of a leaf, a gap is almost invariably produced; the leaf strands attach to the sides of this gap, which is therefore continuous with the leaf gap. In cases of this kind,

the gap in the cylinder above the insertion of a root might be interpreted as the beginning of the leaf gap; but in very many cases openings of an exactly similar nature occur when only a root is involved. These gaps close in the same manner as leaf gaps; for convenience they may be referred to as root gaps.

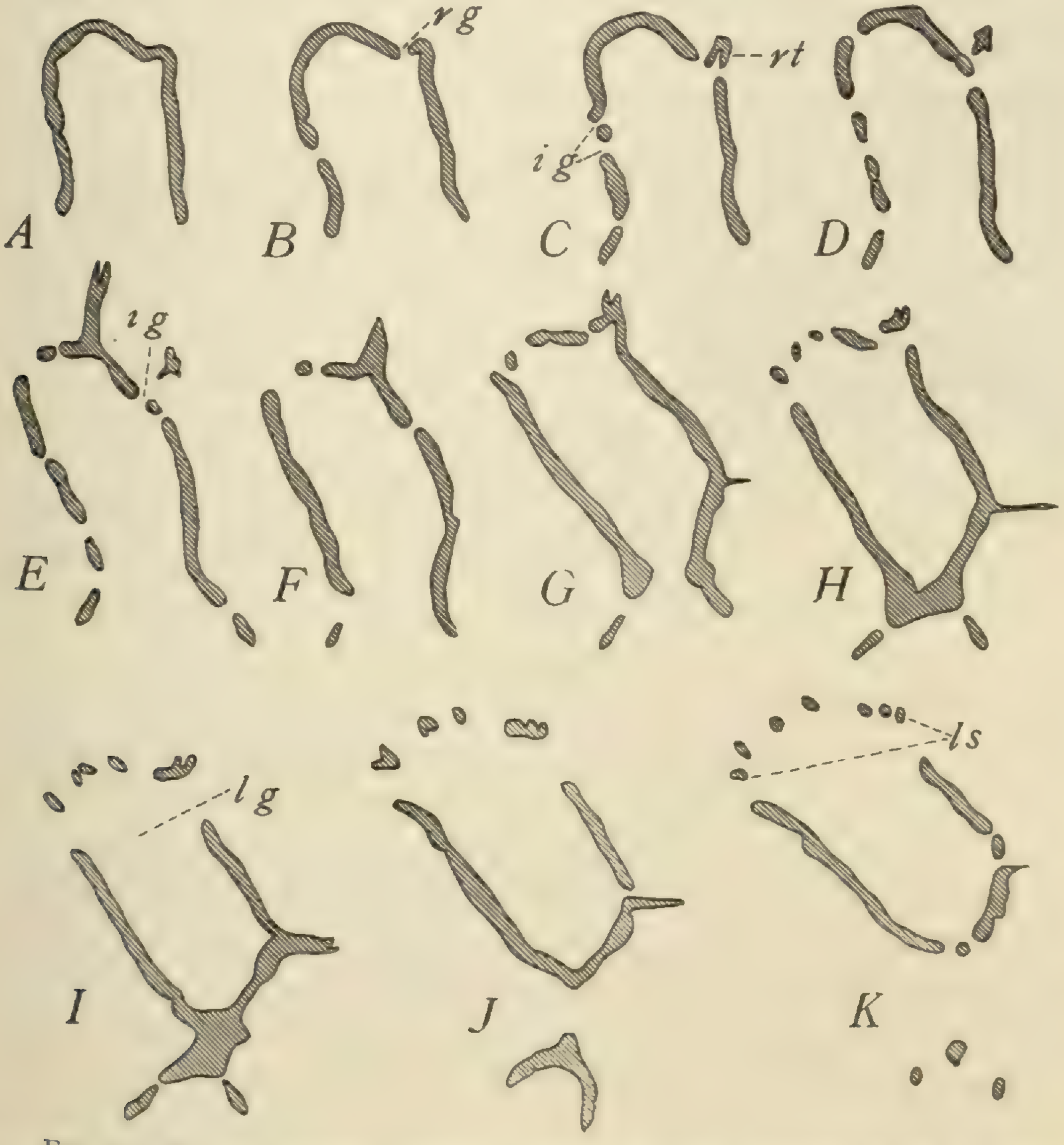

FIG. 11.—Stele of mature rhizome, showing various openings in the cylinder; only the xylem is shown: *rg*, root gap; *rt*, root trace; *ig*, incidental gap; *lg*, leaf gap; *ls*, leaf strands; ×4.5.

In addition to these, openings not related to outgoing strands often occur in the cylinder (fig. 11, *B*–*F*). These incidental gaps sometimes occur at the margin of a leaf gap; a strand separates from the margin of the gap, runs parallel to it for a time, and later

fuses with it. At other times, a long narrow slit may occur in the cylinder. Some of the gaps are relatively large, measuring 0.6× 0.7 mm. Apparent gaps, due to a failure of the xylem parenchyma to lignify, sometimes occur; in these the phloem is not interrupted. In most of the incidental gaps, however, the cortical parenchyma connects with that of the pith, as in leaf and root gaps.

Lignification occurs first in the layer of tracheids nearest the pith; these first xylem elements differ in no way from those outside. They are true tracheids, often very irregular in shape; lobed and even branched forms occur. They are relatively short, 3–6 times as long as broad; the walls are reticulately thickened. The lignification does not begin at definite points, but indiscriminately throughout the inner layer. It proceeds in an outward direction; in the mature stems the xylem is 5 or 6 tracheids in thickness. Occasional irregular divisions occur within the procambium strand after lignification has begun, but there is no true secondary thickening.

The phloem is uniformly a single layer of cells; it is separated from the xylem by a layer of parenchyma 3–5 cells in thickness. There is no endodermis except in the extreme basal region and at the points of attachment of roots; in these instances it is a mere extension of the root endodermis, and is not to be considered as related to the stem stele. The phloem abuts directly upon the cortical parenchyma composed of large spherical or ellipsoidal cells with intercellular spaces; the cells of the layers next the phloem are somewhat smaller than those farther out. The walls of all the cortical cells are secondarily thickened with cellulose, as in the inner region of the root cortex; the pits are much larger. The pith is in all respects similar to the cortex; the cells of the layers next the xylem are considerably smaller than the average. There is no starch storage in any part of the plant, but fats occur in some quantity in all parts, especially in the pith and cortex of the stem. The growth of the stem is by a tetrahedral apical cell, as in other species of the genus; its segmentation was not examined.

The largest rhizome of the Australian material presents an interesting variation of this usual situation. The base has decayed and it is impossible to say whether the stem originated from a

bud or not. In the lowest part, the cylinder is already definitely organized and of the full diameter. There are five gaps corresponding to leaves that were no longer attached, and 18 leaves were still in position. This is almost certainly the oldest specimen secured.

Up to the level of the fourth leaf gap, the stem cylinder agrees in all details with the usual form, as described above; but about midway of the gap, a strand of xylem separates from the margin of the gap, swings over to the center of the opening, broadens, and by connection with the xylem at either side closes the gap. The gap of the sixth leaf is closed by a similar strand. The gaps of the fifth and seventh leaves are closed in a similar manner by strands which arise as procambium in the parenchyma of the openings. At the level of the fourth leaf, a strand separates from the inner surface of the cylinder opposite the point of connection of a root. This strand runs in the pith near the middle of the cylinder for a distance of about 4.5 mm. and finally closes the gap of the eighth leaf in the manner described. In addition to these five strands, there are in this portion of the stem seven others which arise as procambium or by separation from the inner surface of the cylinder and disappear in the same way, without being in any way related to leaf gaps. One of these closes an incidental gap of the cylinder.

From the level of the eighth leaf to that of the twelfth, there are no medullary strands, and the stele presents the usual appearance. At this point another system of seven strands appears; their positions and behavior are shown in fig. 12. Between the seventeenth and twentieth leaves, no medullary strands occur; but at the level of the latter, three more strands appear as procambium in the pith. One appears in the opening caused by the twenty-first leaf and closes that gap; another appears in the center of the base of the twenty-second leaf, and moves inward and closes the gap. The third, arising near the center of the cylinder, branches once or twice; one branch is recognizable as a procambium strand at a short distance from the apical region.

In all, 23 medullary strands occur: 16 arise as procambium in the pith, 5 as branches from the inner surface of the cylinder, and 2 as branches of other strands; 9 of them are concerned in the

closure of leaf gaps, 7 fuse with the inner surface of the cylinder, 4 disappear by fusion with other strands, and 3 disappear by the gradual fading out of the procambium. They range in length from

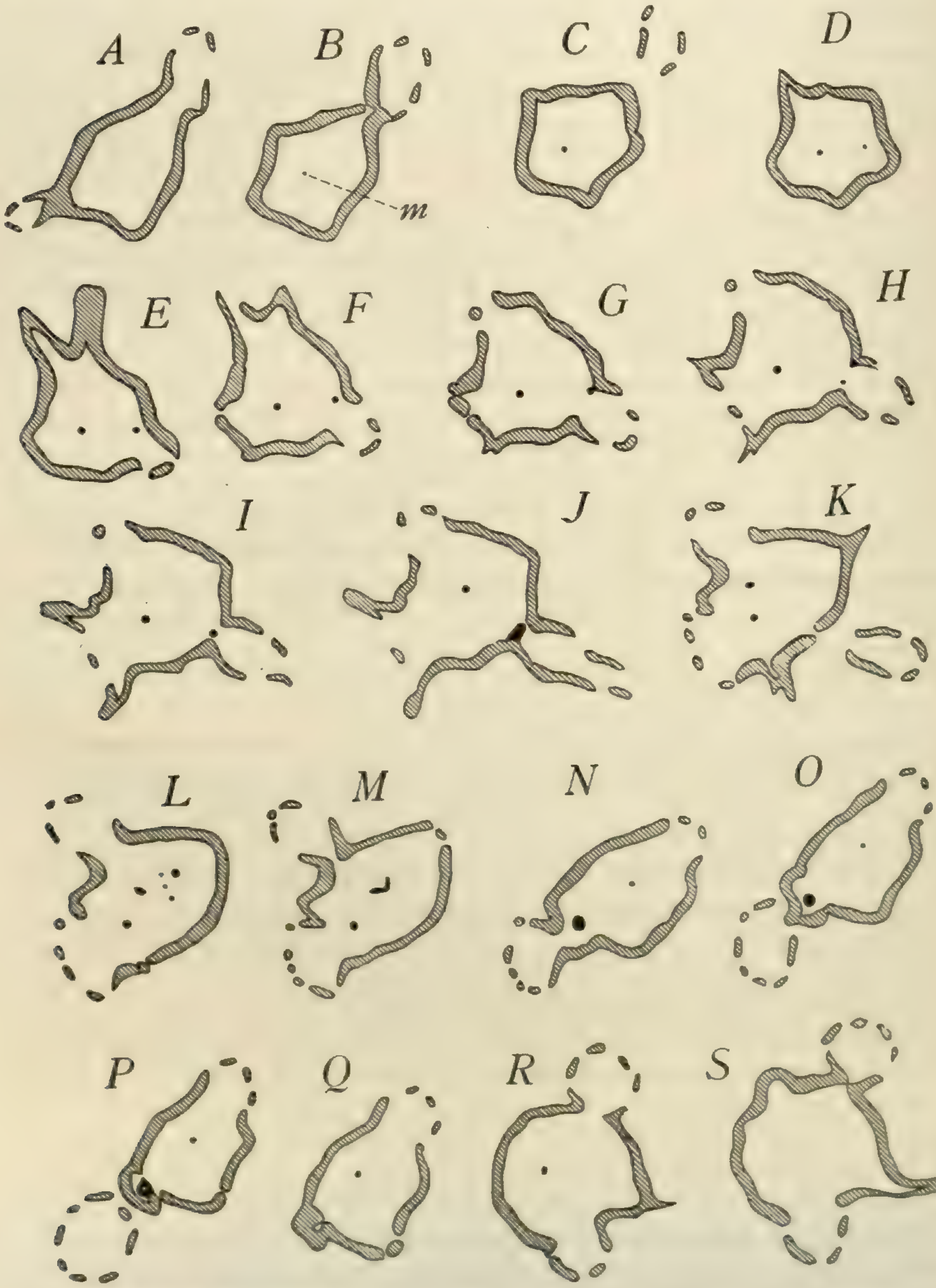

FIG. 12.—Stele with medullary strands; only the xylem is shown: *m*, first medullary strand; ×4.5.

3 or 4 tracheids up to 5.4 mm. in the case of the strand concerned in the closure of the gap of the fifteenth leaf, as shown in fig. 12. A cross-section of one of the strands is shown in fig. 13; they consist of xylem and parenchyma, without a trace of phloem. The largest show 30–40 tracheids in cross-section; 8–12 tracheids is the usual size. No protoxylem can be identified; the tracheids all resemble those of the cylinder.

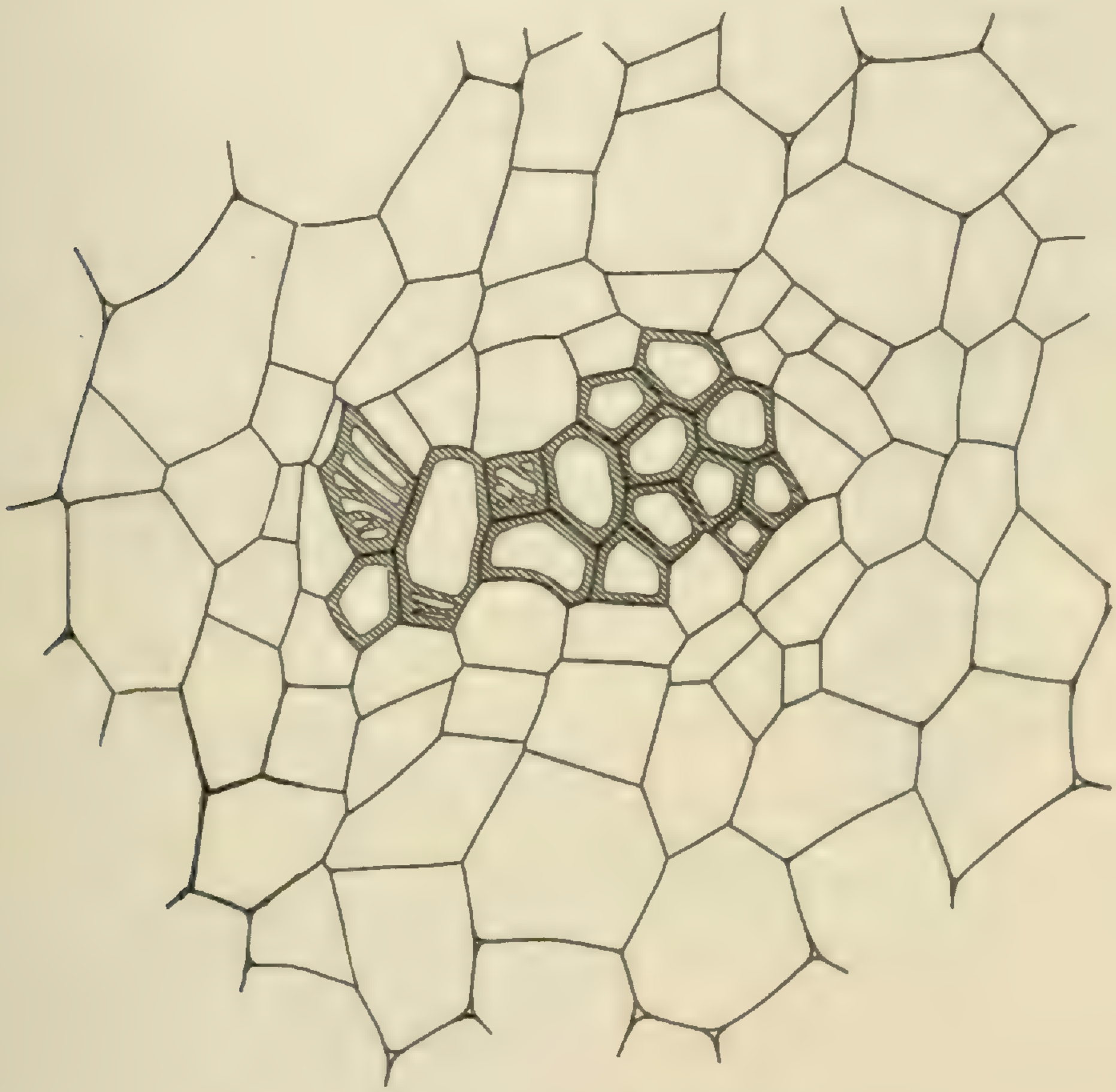

FIG. 13.—Detail of a medullary strand; ×236

In only one other rhizome was anything of this nature observed. This specimen is from Samoa, and has only three leaves; the base is preserved and shows its origin from a bud. At the level of the second leaf a small strand, consisting of a few tracheids only, arises as a procambium at a short distance from the inner surface of the cylinder. It very soon fuses with the cylinder but remains evident as a ridge for a considerable interval. Fron the point of its appearance to that of disappearance is about 0.35 mm.

The leaf

The relation between roots and leaves is very variable. Two or more roots usually appear on the buds before the formation of the first leaf. In the mature stele, roots often connect with the cylinder immediately below a leaf gap; but it is equally common to find two roots attached at the sides of the base of a leaf. No definite relationship between the two can be shown.

As already stated, the leaves in exceptional cases reach a length of 2.7 meters. The stout circular or oval petiole may measure 1 cm. or more in diameter and merges insensibly into the blade; the latter in unbranched leaves is 2–3 cm. in width, but in branched or lobed leaves may reach 5 or 6 cm. at the point of division. Branching and lobing of the leaf occurs very commonly in the Samoan material; in all cases the separation occurs beyond the point of attachment of the fertile spike. There is no absciss layer, as described for *Botrychium virginianum* by JEFFREY (4).

FIG. 14.—Rhizome with young leaf: *s*, fertile spike; ×1.

In the bud, the tip of the leaf is curved over; the fertile spike when first recognizable is attached just beyond the curve, with its tip directed toward the stem. The original development of the leaf is by an apical cell, but its later enlargement is by intercalary growth. As indicated by fig. 14, this begins at the base and proceeds toward the tip. The portion between the spike and the stem may be 4–5 cm. in length when the portion beyond the spike is only 2–3 mm. long. In the mature leaf, the spike is attached at a point about one-third of the way from the base to the tip of the leaf.

The number of strands separating from the cylinder to constitute the leaf trace varies from 3 to 12; 5 is the commonest number. There is a general relation between the size of the leaf. the size of the gap, and the number of strands. The strands usually attach to the cylinder in a circle, the uppermost one after

the leaf gap is actually closed (fig. 12, *P;* fig. 11, *I*); in some cases, however, all the strands connect within the lower portion of the opening in the cylinder.

The general course of the strands in the leaf base is at a slight angle upward from the point of connection with the cylinder; in very large leaves, the lower ones may run horizontally or even in a downward direction. Branching of some of the strands usually occurs within the cortex of the stem, so that as many as 20 strands may be found in the base of the petiole. This may not occur in small sterile leaves, so that a section of the petiole may show as few as 3 strands.

In the sterile leaves, the strands, 3–5 in number, arrange themselves in the shape of a C, with the opening directed adaxially. The strands in the extremities of the arc in passing up through the petiole swing out toward the margins as the blade is formed, and all come to lie in a single plane with xylem adaxially directed. Frequent branching and anastomosing of the strands occur, so that the number may reach 15 or 20 in the blade of the leaf.

In the fertile leaves, the strands, 4–12 in number, are arranged in a circle or oval as they leave the cylinder, and they maintain this arrangement through the petiole. They branch and anastomose freely, forming a complete cylindrical network; no strands connect across the circle. As the petiole broadens and flattens, the strands arrange themselves in two series: the outer, consisting of 10–15 strands with xylem directed adaxially, form the vascular system of the blade; the inner series, of 5–8 strands with xylem abaxially directed, is reduced by fusions to 4–6 strands which form the vascular supply of the spike. Anastomosing and branching occur as before within each series, but there is no further connection between the two systems. Beyond the point of connection of the fertile spike, the single series of strands constituting the reticulate veining of the blade may increase to as many as 30 in branching leaves. They form a closed system with the exception of a very few small branches which end blindly in the tip.

In the leaf strands as they separate from the cylinder, the xylem elements are all alike, but in the base of the petiole and throughout the leaf the first formed elements can be distinguished by their

smaller size. These protoxylem elements are spiral vessels; they always occur at the inner margin of the xylem, that is, in the endarch position. The protophloem, consisting of 3 or 4 cells, develops at the opposite limit of the strand; the later developed phloem of 6–10 cells is arranged in 2 or 3 layers. One or two layers of parenchyma separate the xylem and phloem in a mature bundle (fig. 15).

In the mature strands of the blade, and, to a less extent, of the petiole, a definite bundle sheath is developed by the thickening of the walls of 2 or 3 layers of parenchyma surrounding the vascular elements (fig. 15). The thickening material is cellulose and the walls are pitted as in similar tissues of the stem and root. This bundle sheath is separated from the protoxylem by 2 or 3 cells of parenchyma, but borders directly upon the protophloem. As a consequence of growth within the bundle sheath, the protophloem in a mature strand is crushed against and between the cells of the sheath.

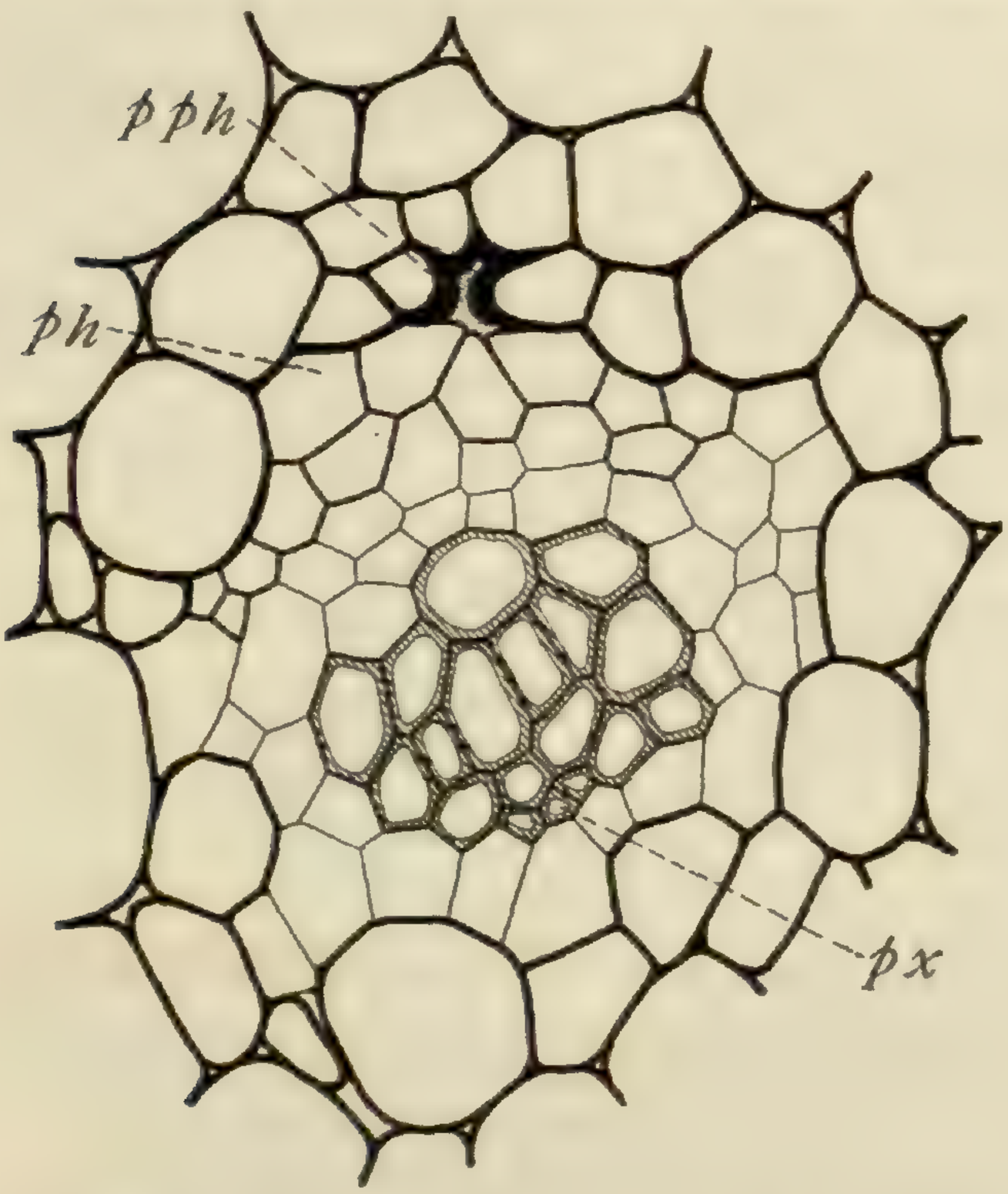

FIG. 15.—Detail of a leaf strand: *pph*, protophloem; *ph*, metaphloem; *px*, protoxylem; ×236.

The spike

As stated above, the strands of the leaf with xylem directed abaxially form the vascular supply of the spike. At the base of the peduncle these are 4–6 in number; they continue with occasional anastomosing and fusion through the median portion of the spike. At the base of the fertile portion of the spike, the strands at the margin of the median region run immediately at the base

of the sporangia. Farther up the spike, where the ridge of sterile tissue which extends into the spore mass is well developed, the strand occupies a position well within this ridge (fig. 16, *B*). There is no branching of the marginal strand below the first few sporangia; but between the third and fourth sporangia, a short lateral branch extending halfway to the edge of the spike usually occurs. Similar strands occur between all the sporangia above this point (fig. 16, *A*). They consist in cross-section of 10 or 12 tracheids, and occupy the center of the thin wall separating adjacent sporangia. Near the margin of the spike they spread out in the shape of a fan and end blindly (fig. 16). The central strands are reduced by fusion to a single strand when the tip of the spike is reached. Between the terminal sporangia this splits into two strands which run out above the sporangia and end in the fan-shaped arrangement described above.

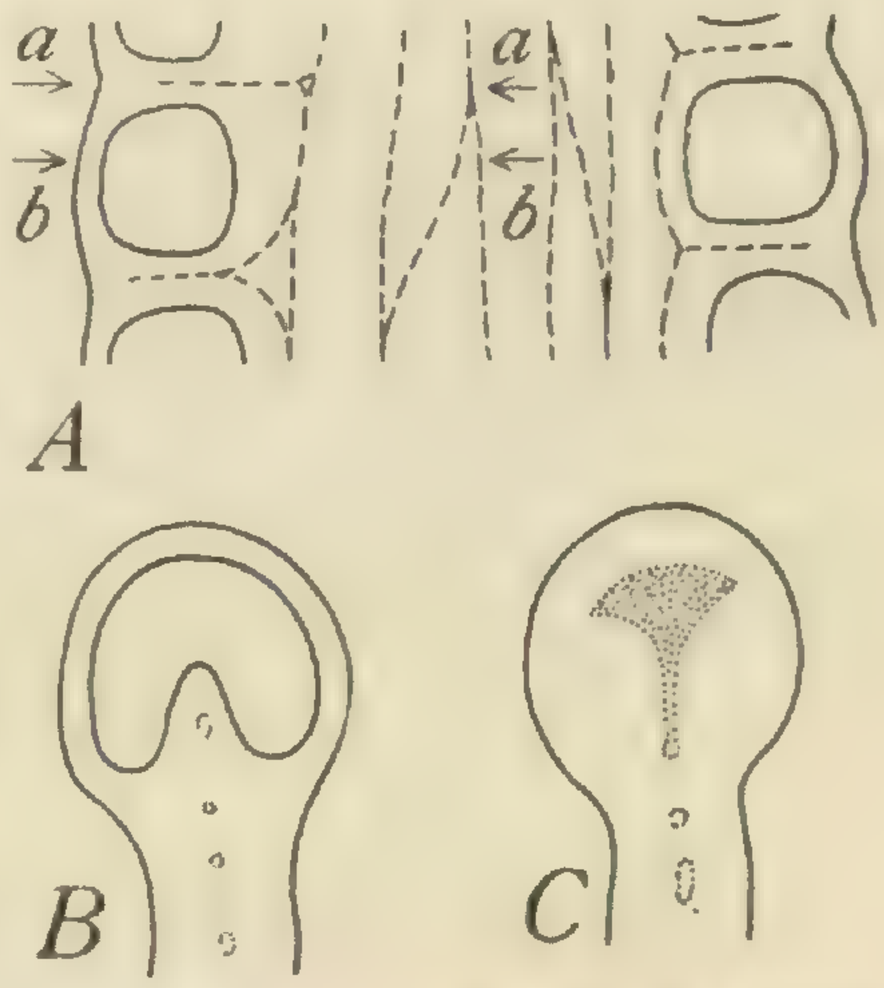

FIG. 16.—Diagrams of vascular system of the spike: *A*, median longitudinal section; *B*, transverse section in plane *bb*; *C*, transverse section in plane *aa*; ×4.5.

The strands of the peduncle and of the median region of the spike show the same structure as those of the blade of the leaf, but the bundle sheath is much less developed. In the strands between the sporangia, the bundle sheath is absent. The protophloem is on the adaxial side of the xylem, but the later developed phloem elements may extend in the shape of a U about the xylem, which is always endarch. In the fan-shaped portion of the strands the phloem cannot be distinguished; there is no distinction between protoxylem and metaxylem.

Discussion

The most striking characteristic of the anatomy of this species is the extreme variability of certain structures. The number of protoxylem strands of the root and the number of strands constituting the leaf trace vary almost directly with the size of the organs concerned. The external conditions which determine whether the

stems are large in diameter or small determine in the same way the number of the strands of the leaf. Such variations may be considered as produced directly by growth conditions and therefore of physiological interest only.

The variability in the connection of the stem stele with that of the root is related to the position in which the bud develops; thus, the bud which developed the stem stele shown in fig. 7 was located directly over one of the protoxylem strands of the root, while that of the specimen shown in fig. 8 was placed midway between two such strands. In the same way, the condition represented in fig. 9 may be ascribed to a more gradual divergence of the root and stem axes; for a time, the two apical cells formed a common tissue within which a single stele was developed, as in fig. 10. When the angle between the two axes became greater, separate steles were developed.

It is of course impossible to say what determines these relations of position of the protoxylem strands and the stem meristem, or the rate of divergence of the two axes; but it seems evident from these variations that the manner of development of the stem stele in a bud is controlled by chance or external conditions. Hence we may conclude that the stelar characters shown in the development from buds cannot be used in any discussion of phylogeny. It is to be noted, however, that two features are constant; these are the collateral arrangement of the stelar elements and the endarch position of the protoxylem.

The occurrence of occasional strands of xylem in the pith is a feature that is unique, so far as the writer is aware. The manner of origin of these strands and their behavior suggests somewhat the medullary strands of *Marattia;* but the absence of phloem distinguishes sharply between the two cases. In any consideration of this feature it should be borne in mind that these strands occur to any considerable extent in only a single specimen.

LANG (5) from a study of *Botrychium Lunaria* has concluded that the pith of Ophioglossaceae is purely intrastelar in origin. This opinion is based in part upon the occurrence of scattered tracheids in the pith, particularly in injured rhizomes; this formation of xylem elements from parenchymatous cells of the pith is considered strong evidence of the stelar origin of the pith. The medul-

lary strands of *O. pendulum* afford a much more definite case of this sort; they are in no way a traumatic response; cells of the pith develop a procambium which develops into xylem only; this may be taken to indicate that all cells of the pith are potentially xylem-producing, and therefore stelar.

Summary

1. The root stele varies from diarch to hexarch. The roots branch monopodially, and the branches are diarch at the base.

2. Buds develop upon the roots in the same manner as in *O. vulgatum*.

3. The rhizomes are always radial in structure; the leaves are inserted in an irregular spiral.

4. The connection of the stem stele with that of the parent root is very variable; no phylogenetic significance can be attached to the details of development of the stem stele of a bud.

5. The stele of a mature rhizome is an ectophloic siphonostele with large overlapping leaf gaps; there is no secondary thickening. Root gaps usually occur above the points of insertion of root strands. Incidental gaps not related to outgoing strands occur commonly.

6. In a single large rhizome, numerous xylem strands occur within the pith. These arise from the inner surface of the cylinder or as procambium; some of them are concerned in the closure of leaf gaps, and others disappear as procambium or by fusion with the cylinder. They consist of xylem only.

7. The vascular supply of the leaf consists of 3–12 strands, the number varying with the size of the leaf base. These strands form a cylindrical network in the petiole; in the lower portion of the blade, they constitute two series of strands with xylem oppositely directed. The strands with xylem abaxially directed form the vascular supply of the spike.

The writer is indebted to Professor JOHN M. COULTER for many suggestions and criticisms; and to Dr. CHARLES J. CHAMBERLAIN and Dr. W. J. G. LAND for the material used and for direction during the investigation.

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SOME EFFECTS OF COLLOIDAL METALS ON SPIROGYRA[1]

W. D. HOYT

(WITH FOUR FIGURES)

The effects of colloidal metals on the activities of living cells seem to have received but little attention, although it appears that studies of such effects should yield results of importance to protoplasmic physiology. We regard protoplasm as largely composed of colloidal material, and believe the enzymes affecting many of the protoplasmic processes to be colloidal in their nature. Further, we know that, outside of protoplasm, colloids affect one another in many different ways, and that colloidal metals are capable of bringing about reactions which are similar, in many respects, to those usually related to the action of enzymes. It becomes a matter of considerable interest, therefore, to determine the effects of colloidal metals on protoplasm and protoplasmic products, especially since the soluble salts of many metals are known to be extremely poisonous. The phase of the problem thus suggested with which the present paper has to deal may be stated as follows: Solutions of the salts of silver, gold, and platinum are poisonous to protoplasm; colloidal solutions of the metals named may bring about reactions in non-living materials similar to those caused by enzymes; what, then, may be the effects of such colloidal solutions on protoplasm?

The present investigation was planned to obtain some preliminary information bearing upon the answer to this question. It has mainly to do with the descriptive aspect of some of the superficial effects of colloidal solutions of platinum, gold, and silver on two species of *Spirogyra*, with a few notes on other algae. The work was undertaken at the suggestion of Professor GEORG KLEBS, and was carried out in the Botanical Institute of the University of

[1] Botanical contribution from the Johns Hopkins University, no. 30. These investigations were carried on while the author held the Adam T. Bruce Fellowship of the Johns Hopkins University. A summary of the results was presented before the Botanical Society of America at its Washington meeting, December 28, 1911.

Heidelberg. Unless otherwise stated, the species used was *Spirogyra longata* (Vauch.) Kg. The material and general methods were the same as those described in a previous paper (8). The original culture of *Spirogyra longata* was brought from Algiers, but the alga had been growing for nearly three years in the laboratory, in a stock culture which received small additions of tap water from time to time. The filaments of this stock culture were apparently healthy and of the usual appearance. The colloidal solutions of silver, gold, and platinum here employed were made with non-toxic water, according to the method previously described, of distillation in glass with animal charcoal present in the retort. They were kindly prepared for the author by Dr. W. FRAENKEL in the laboratory of Professor G. BREDIG at the University of Heidelberg, by atomizing the metals with an electric arc under water. This method has been described by BREDIG (**2, 3, 4**). The solutions were kept in flasks of Jena glass. Those of gold and platinum were determined by Dr. FRAENKEL and found to contain 90 ppm. (parts per million) of gold and 96 ppm. of platinum, respectively. The sample of the silver solution was unfortunately lost before being determined, but, as all three solutions were originally prepared so as to contain the same amounts of metal (with an error not greater than 10 ppm.), the concentration of the silver solution here employed may be considered as approximately 90 ppm. The solution of gold was purple in color, that of platinum was yellow-brown, while the solution of silver was grayish brown. The gold solution, on standing, formed a deposit, probably of the larger particles, on the walls both of the flask containing the stock solution and of the culture dishes, but the intensity of its color was not perceptibly diminished by this deposition. The solutions of silver and platinum gave no observable deposits. In considering the results obtained with these solutions, the possibility is not to be forgotten that small amounts of metal oxides may have been formed in the process of preparation, and that the solutions are not to be considered as necessarily and entirely free from metal in the ionic condition.

Colloidal solutions of silver, gold, and platinum prepared by BREDIG, similar to those used by the author except that they were weaker and were not made with carbon-distilled water, were

examined by ZSIGMONDY (17) with the ultra-microscopic apparatus devised by him. From his results it was calculated that the diameters of the particles in the silver solution were about 50–77 $\mu\mu$. The gold solution contained particles 20–80 $\mu\mu$ in diameter, the larger ones being separable by filtration. There was a much greater range in the size of the suspended platinum particles, but by far the greater portion of these were very small and had an average diameter of 44 $\mu\mu$. As the solutions employed in the present investigation resembled in appearance those described and examined by ZSIGMONDY, it is probable that the particles in these solutions had a similar degree of magnitude.

The results upon toxicity obtained in the present investigation are shown in tables I–IV, which are self-explanatory. In the following consideration of the main points the Roman numerals in parentheses refer to the tables and the Arabic ones to the experiment numbers as there given.

The solution of silver was extremely toxic (I, 1), killing the alga within 17 hours in concentrations above 0.045 ppm. (0.05 per cent of the solution originally prepared) and injuring many of the filaments in strengths as low as 0.00225 ppm. (0.0025 per cent of the original solution). In filaments thus killed the cell contents were often dark and disorganized and were more or less contracted.

Various substances were added to the colloidal silver solution to determine the effect of their presence upon its toxicity. The addition of salts to form a 0.5 per cent concentration of Crone's nutrient solution[2] to colloidal solutions of silver in concentrations of 0.045 ppm. or less (I, 2), produced marked improvement, as did also the addition of about 81 ppm. colloidal platinum (I, 3), or of about 0.1 gr. animal charcoal (I, 4). Solutions which had previously been injurious or fatal were thus changed into solutions which were entirely or almost entirely non-injurious during the period of the experiments (1–3 days). In such cases the cell contents retained their usual healthy appearance.

The effect of the gold solution shows that it was much less toxic than that of silver. In order to obtain colloidal gold and hold it in

[2] This solution contained salts in the following proportions: 1 gr. KNO_3; 0.5 gr. $MgSO_4$; 0.5 gr. $CaSO_4$; 0.25 gr. $Ca_3(PO_4)_2$; 0.25 gr. $Fe_3(PO_4)_2$.

solution, about 0.02 per cent of sodium hydroxide was added to the water with which the colloidal solution was prepared, so that this solution contained NaOH. Two experiments were carried out with

TABLE I

CULTURES OF SPIROGYRA LONGATA IN COLLOIDAL SILVER SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Silver in medium, approximately, ppm.	Other substances in medium, approximate amounts	Condition of plants*	Duration of experiment, days*
1*a*	90.0	.....	D, D	1, 1
1*b*	45.0	.....	D	1
1*c*	22.5	.....	D	1
1*d*	4.5	.....	D	1
1*e*	0.9	.....	D	1
1*f*	0.108	.....	D	1
1*g*	0.045	.....	P, D	1, 1
1*h*	0.0135	.....	E	1
1*i*	0.009	.....	Gp	3
1*j*	0.0045	.....	E	1
1*k*	0.00225	.....	G, D	3, 1
2*a*	0.045	Salts to form 0.5 p.c. Crone's solution	E, Eg	2, 1
2*b*	0.0135	*do.*	E	1
2*c*	0.009	*do.*	Eg	3
2*d*	0.0045	*do.*	E	1
3*a*	0.045	Colloid. Pt, 81 ppm.	E, E	2, 1
3*b*	0.0135	*do.*	E	1
3*c*	0.009	*do.*	E	3
3*d*	0.0045	*do.*	E	1
4*a*	0.045	Animal charcoal, 0.1 gr.	E, G	2, 1
4*b*	0.0135	*do.*	E	1
4*c*	0.009	*do.*	E	3
4*d*	0.0045	*do.*	E	1

*In tables I–IV the condition of the plants is indicated as follows: *E*, *excellent*, meaning that none of the filaments seemed injured; *G*, *good*, meaning that less than half seemed injured; *P*, *poor*, meaning that more than half seemed injured; *D*, *dead*, meaning that all or practically all the filaments were dead. A combination of letters denotes a condition between those denoted by the combined letters, respectively; thus, *Eg* means a condition between *excellent* and *good*, etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the last column (duration in days) refer to the experiments in the same order as do the letters; thus, the first number denoting *duration* and the first letter denoting *condition* both refer to the same experiment, etc.

Spirogyra from the stock culture, filaments being transferred to the undiluted, alkaline solution of colloidal gold (II, 5*a*). In one case the filaments remained in excellent condition during the period of the experiment (2 days); in the other case, the alga remained with-

out apparent injury for 5 days, except that the chloroplasts dwindled slightly; but on the sixth day, when the experiment was discontinued, many filaments were dead. For comparison with the above, two experiments were performed with *Spirogyra* which had grown

TABLE II

CULTURES OF SPIROGYRA LONGATA IN SODIUM HYDRATE SOLUTION, WITH AND WITHOUT COLLOIDAL GOLD AND OTHER ADDITIONS

Culture no.	Concentration of NaOH, per cent*	Other substances in medium, approximate amounts, ppm.	Condition of plants	Duration of experiment, days
5*a*†	0.02	Colloid. Au, 90.0	E, Eg	2, 6
5*b*	0.02 [Plants from 0.1 p.c. Crone's sol., 72 days old]	Colloid. Au, 90.0	Eg, P	3, 4
5*c*	0.01	Colloid. Au, 45.0	Gp	2
6	0.02	.....	D	1
7	0.01	.....	Pd	5
8	0.01	Colloid. Pt, 48.0	E	5
9*a*	0.02	Colloid. Ag, 0.0135	D	1
9*b*	0.02	Colloid. Ag, 0.009	Pd	1
9*c*	0.02	Colloid. Ag, 0.0045	Gp	1
10	0.02	.....	Eg, D	1, 1
10*a*	0.01	.....	E, Eg	1, 1
11*a*	0.02	$AuCl_2$, 100.0	P, D	1, 1
11*b*	0.01	$AuCl_2$, 100.0	Eg, Gp	1, 1
12*a*	0.02	Colloid. Pt, 86.4	E, G	1, 1
12*b*	0.01	Colloid. Pt, 86.4	E, E	1,1

*The sodium hydrate solution used in cultures 5*a*–9*c* was a portion of that employed in the preparation of the colloidal gold solution; that used in cultures 10–12*b* was prepared at another time. The strength of the former solution is given only approximately, that of the latter is given accurately.

† Unless otherwise noted, the material of *Spirogyra* used in these experiments was from the stock culture.

in 0.1 per cent Crone's solution for 72 days. The filaments were rinsed in non-toxic water and placed in the undiluted gold solution (II, 5*b*). In one case, the filaments remained in excellent condition two days, but a few showed injury on the third day; in the other case, the alga remained in excellent condition one day, but on the

TABLE III

CULTURES OF SPIROGYRA LONGATA IN COLLOIDAL PLATINUM SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Platinum in medium, approximately, ppm.	Other substances in medium, approximate amounts, per cent	Condition of plants	Duration of experiment, days
13	96.0	.....	Eg, E	22, 3
14*a*	86.4	KCl, 0.1	P	12
14*b*	.....	*do.*, control to 14*a*	Pd	6
15*a*	86.4	$MgSO_4$, 0.02	Gp	19
15*b*	.....	*do.*, control to 15*a*	Pd, D	6, 2
16*a*	48.0	Tap water, 50.0	G	20
16*b*	.....	*do.*, control to 16*a*	Pd	12
16*c*	48.0	Ord. dist. H_2O, 50.0	Gp	26
16*d*	.....	*do.*, control to 16*c*	D, D, D	2, 1, 1
17	.....	$PtCl_4$, 0.008 $MgSO_4$ 0.02	Pd	12

TABLE IV

CULTURES OF SPIROGYRA DECIMINA (NOS. 18–23*b*) AND OF OSCILLATORIA (NO. 24) IN SODIUM HYDRATE SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Concentration of NaOH, per cent*	Other substances in medium, approximate amounts, ppm.	Condition of plants	Duration of experiment, days
18	0.02	Colloid. Au, 90.0	Pd	4
19	0.01	.....	Pd	2
20	0.01	Colloid. Pt, 48.0	E	2
21*a*	0.02	.....	Eg	1
21*b*	0.01	.....	E, Eg	1, 1
22*a*	0.02	$AuCl_2$, 100.0	Eg	1
22*b*	0.01	*do.*	Eg, Gp	1, 1
23*a*	0.02	Colloid. Pt, 86.4	E	1
23*b*	0.01	Colloid. Pt, 86.4	E, E	1, 1
24†	0.02	Colloid. Au, 90.0	E, E	4, 3

* The sodium hydrate solution used in cultures 18–20 and 24 was a portion of that employed in the preparation of the colloidal gold solution; that used in cultures 21*a*–23*b* was prepared at another time.

† The alga used in no. 24 was *Oscillatoria* from a 0.1 per cent Sachs's solution, thickened with 1 per cent agar.

second and third days many filaments were injured, while on the fourth day practically all were dead.

Diluting the gold solution with an equal volume of non-toxic water produced no improvement. In such a solution *Spirogyra* from the stock culture remained in excellent condition one day, but on the second day about half of the filaments were dead (II, 5*c*).

The effect of transferring portions of *Spirogyra* from colloidal gold solution to non-toxic distilled water was tested. Filaments which had been in gold solution for two days, when placed in this water, showed injury within one day and were, for the most part, dead within two days, while the portions of the alga remaining in the gold solution were still in excellent condition. Other effects of such transfers will be described later.

Although the colloidal gold solution itself was only slightly injurious, the sodium hydrate solution (about 0.02 per cent) used in its preparation was extremely toxic, killing all the filaments and causing contraction of their cell contents within 17 hours (II, 6). This alkaline water, when diluted with an equal volume of non-toxic distilled water, was still decidedly toxic (II, 7), but was not injurious during the period of the experiment (5 days) when diluted with an equal volume of the full strength colloidal platinum solution (II, 8). A similar improvement by the addition of colloidal platinum was produced on 0.02 per cent and 0.01 per cent solutions of NaOH prepared at another time (II, 10, 12). Even weak solutions of silver seemed to produce a beneficial effect on this alkaline solution, since the alga lived better with the addition of 0.01–0.005 per cent of the full strength silver solution (0.009–0.0045 ppm.) than in the NaOH solution alone (II, 9). The addition, however, of 0.01 per cent of $AuCl_2$ to 0.02 per cent and 0.01 per cent solutions of NaOH produced no improvement in these solutions; on the contrary, the soluble gold salt seemed to make the NaOH solutions more injurious (II, 10, 11).

The colloidal solution of platinum was still less injurious than that of gold, filaments of *Spirogyra* placed in this solution remaining in apparently perfect condition for 9 days and dying only after 22 days (III, 13). Not only was this platinum solution not injurious during the first few days of the experiments, but it produced

improvement in a 0.1 per cent solution of KCl (III, 14), in a 0.02 per cent solution of $MgSO_4$ (III, 15), and in a mixture with an equal volume of tap water or an equal volume of ordinary distilled water (III, 16), over such solutions without the colloidal platinum. It also, as has been shown above, produced improvement in a weak colloidal solution of silver and in a solution of NaOH. The addition, however, of 0.008 per cent $PtCl_4$ to a 0.02 per cent solution of $MgSO_4$ produced no improvement in this solution (III, 17).

A few experiments were made with other algae. *Spirogyra decimina* Kg.,[3] newly brought to the laboratory, gave practically the same results as those already described, except that it was more quickly injured by colloidal gold solution (IV, 18–23). However, filaments of a species of *Oscillatoria* growing in 0.5 per cent Sachs's solution thickened with 1 per cent agar, when transferred to the gold solution remained in perfect condition and continued their usual movements during the period of the experiment (3–4 days) (IV, 24).

FIG. 1.—*Spirogyra longata* from stock culture, 24 hours after transfer to colloidal gold solution; examined and drawn in gold solution.

When filaments of *Spirogyra longata* from the stock culture or filaments of *S. decimina* were placed in colloidal gold solution, a striking phenomenon was observed; the outer layer of the cell walls swelled irregularly within 17 hours, forming gelatinous sheaths over considerable portions of the external surfaces (fig. 1). These sheaths, whether greatly swollen or not, were colored purple by the gold solution. The cells remained in otherwise excellent condition during this time; their contents were uncontracted and normal in appearance, and the chloroplasts retained their usual green color. That the cells were alive and healthy was indicated by the fact that they were easily plasmolyzed by glycerine. When filaments of *S. longata* were examined directly in the gold solution, they showed slight irregular swellings (fig. 1), but when transferred from the gold solution to glycerine solution or to non-toxic distilled water marked swelling became evident over the entire wall within one or

[3] Determined from O. KIRCHNER, Die mikroskopische Pflanzenwelt des Süsswassers. 1885.

two minutes (figs. 2, 3). Under the latter conditions the modified outer layer frequently became ruptured, breaking away from the wall in purple, gelatinous masses and leaving the remainder of the wall and the other portions of the cell uncolored and apparently unaffected. Filaments of *S. decimina* formed swollen sheaths in the gold solution itself similar to those formed by *S. longata* when transferred from this solution to water. After a

FIG. 2

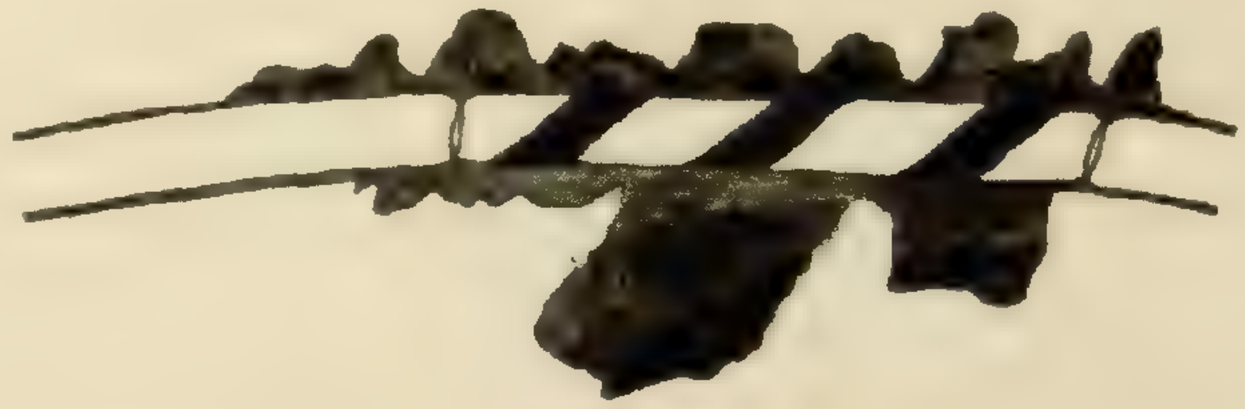

FIG. 3

FIGS. 2 and 3.—*Spirogyra longata* from same material as that shown in fig. 1, 48 hours after transfer to colloidal gold solution; examined and drawn in distilled water.

sheath had broken from a portion of the wall, no new sheath was formed, the rest of the wall remaining unswollen and uncolored during the period of the experiments (2–6 days). The results of further studies upon the formation of these gelatinous sheaths are presented in table V. Where difficulty was experienced in determining by simple microscopic observation whether or not swelling of the outer wall had occurred, such difficulty was removed by treatment of the material with Bismarck brown, which so stains the unswollen and swollen portions of the walls as to render even very thin sheath layers clearly discernible.

FIG. 4.—*Spirogyra longata* from culture (72 days old) in 0.1 per cent Crone's solution, 48 hours after transfer to colloidal gold solution; examined and drawn in distilled water.

Sheaths were formed in the gold solution by *Spirogyra longata* from the stock culture (V, 1*a*), and by *S. decimina* (V, 1*c*), and they were produced to a very slight degree by a species of *Oscillatoria* (V, 1*d*). No sheaths were thus formed upon filaments of *S. longata* (fig. 4) which had been for 72 days in 0.1 per cent Crone's solution (V, 1*b*), nor by a species of *Hormidium* (V, 1*e*). Sheaths failed to appear upon *S. longata* in colloidal gold solution diluted with an equal volume of non-toxic water (V, 2), nor were they evident upon

TABLE V

VARIOUS TREATMENTS PRODUCING EXTERNAL SHEATHS ON ALGA CELLS, TOGETHER WITH COMPARATIVE TREATMENTS FAILING TO PRODUCE SUCH SHEATHS

CULTURE NO.	ALGA EMPLOYED	MEDIUM EMPLOYED, NON-TOXIC DISTILLED WATER CONTAINING		NUMBER OF TESTS IN WHICH SHEATHS WERE		NOTES*
		Suspensoids and their approximate concentration, ppm.	Solutes and their approximate concentration, p.c.	Formed	Not formed	
1*a*	*Spirogyra longata*†	Gold, 90.0	NaOH, 0.02‡	2	.....	E; sheaths formed within 17 hours
1*b*	*Do.*, from culture in 0.1 p.c. Crone's sol. 72 days old	*do.*	*do.*	.....	2	G
1*c*	*Spirogyra decimina*§	*do.*	*do.*	1	.....	D; within 4 days. Sheaths formed within 17 hours
1*d*	*Oscillatoria*‖	*do.*	*do.*	1 (?)	1	E; a few filaments in one culture showed slight sheaths when transferred to tap water; no sheaths apparent in gold solution. Usual movements observed
1*e*	*Hormidium*	*do.*	*do.*	.....	1	.....
2	*Spirogyra longata*	Gold, 45.0	NaOH, 0.01‡	.....	1	.....
3*a*	*Do.*	.....	$AuCl_2$, 0.01; NaOH, 0.02¶	.....	2	D; many filaments
3*b*	*S. decimina*	.....	*do.*	.....	1	G
4*a*	*S. longata*	.....	$AuCl_2$, 0.01; NaOH, 0.01¶	.....	2	G
4*b*	*S. decimina*	.....	*do.*	.....	2	G
5	*S. longata*	.....	NaOH, 0.02‡	.....	1	D
6*a*	*Do.*	.....	NaOH, 0.01‡	.....	1	D
6*b*	*S. decimina*	.....	*do.*	1	.....	No sheaths after 17 hours, but some colorless ones appeared on second day, when filaments mostly D
7*a*	*S. longata*	.....	NaOH, 0.02¶	.....	2	G, in one culture D, in other
7*b*	*S. decimina*	.....	*do.*	.....	1	D
8*a*	*S. longata*	.....	NaOH, 0.01¶	.....	2	G

TABLE V—*Continued*

Culture no.	Alga employed	Medium employed, non-toxic distilled water containing		Number of tests in which sheaths were		Notes*
		Suspensoids and their approximate concentration, ppm.	Solutes and their approximate concentration, p.c.	Formed	Not formed	
8*b*	*S. decimina*	.....	NaOH, 0.01¶	.....	2	G
9	*S. longata*	Platinum, 96.0	.....	.....	1	E, for 9 days D, after 22 days
10	*Do.*	Platinum, 48.0	Tap water, 50.0	.....	1	G
11*a*	*Do.*	*do.*	NaOH, 0.01‡	.....	1	E
11*b*	*S. decimina*	*do.*	*do.*	1	.....	E; heavy sheaths within 17 hours, colored brown by Pt.
12*a*	*S. longata*	Platinum, 86.4	NaOH, 0.02¶	1	1	G; few slight sheaths in one culture
12*b*	*S. decimina*	*do.*	*do.*	1	.....	E; heavy sheaths within 17 hours; colored by Pt.
13*a*	*S. longata*	*do.*	NaOH, 0.01¶	.....	2	E
13*b*	*S. decimina*	*do.*	*do.*	2	.....	E; heavy sheaths within 17 hours, colored by Pt.
14	*S. longata*	Silver, 90, and various lower concentrations	.....	.....	19	Mostly D, in some cases G
15*a*	*Do.*	Silver, 0.135	NaOH, 0.02‡	.....	1	D
15*b*	*Do.*	Silver, 0.09	*do.*	.....	1	Mostly D
15*c*	*Do.*	Silver, 0.045	*do.*	.....	1	Gp
16	*Do.*	Platinum, 96.0; silver, various concentrations below 90.0	.....	.....	5	E
17	*Do.*	Silver, various concentrations below 90.0	Salts to form Crone's solution, 0.5	.....	5	E

* The general condition of the filaments is here denoted by the symbols E, G, etc., as in previous tables.
† All *Spirogyra longata* used was from the stock culture unless otherwise noted.
‡ Sodium hydrate solution employed in the preparation of the colloidal gold solution.
§ The *Spirogyra decimina* was newly brought from the open.
. The *Oscillatoria* was used from a culture in a 0.5 per cent Sachs's solution thickened with 1 per cent agar.
¶ Sodium hydrate solution as used in preparation of colloidal gold solution, but prepared at another time.

either species of *Spirogyra* in a 0.01 per cent solution of $AuCl_2$ which contained also 0.02 per cent or 0.01 per cent NaOH (V, 3, 4). When filaments of *S. longata* were subjected to the action of the sodium hydrate of the gold solution (approximately 0.02 per cent concentration of NaOH), in the absence of the colloidal gold, no swellings were developed (V, 5). Diluting the sodium hydrate solution by adding an equal volume of non-toxic water was without effect (V, 6*a*). *S. decimina*, however, produced some colorless sheaths on the second day in this 0.01 per cent solution of NaOH, though the majority of the filaments were by this time dead. No sheaths were evident on the first day (V, 6*b*). In sodium hydrate solutions with concentrations of 0.02 and 0.01 per cent, respectively, prepared at another time and perhaps not exactly similar to the solution in which the colloidal gold was formed, neither species of the alga exhibited any swellings (V, 7, 8).

With colloidal platinum no sheaths were formed by *Spirogyra longata* in the undiluted solution (V, 9), nor were they evident in this solution diluted with an equal volume of tap water (V, 10) or with an equal volume of a 0.02 per cent solution of NaOH (V, 11*a*). In this alkaline platinum solution *S. decimina*, on the contrary, formed heavy sheaths within 7 hours, these being colored a deep brown by the platinum (V, 11*b*). Similarly *S. longata* gave only slight sheaths on but a few filaments in slightly-diluted platinum solution containing 0.02 per cent of NaOH; it failed to produce any sheaths at all in platinum solution to which 0.01 per cent of NaOH had been added (V, 13*a*). *S. decimina* formed heavy brown sheaths in both of the last named solutions (V, 12*b*, 13*b*).

No sheaths appeared upon *Spirogyra longata* in any strength of silver solution here tested, either with (V, 15) or without (V, 14) NaOH. Nor were sheaths formed by this species in any strength of silver to which was added the undiluted platinum solution (V, 16) or inorganic salts to form 0.5 per cent Crone's solution (V, 17). In these solutions the filaments remained in excellent condition throughout the experiments.

When no swelling became apparent, as in the presence of colloidal platinum alone or in that of colloidal platinum and colloidal silver together, the cell walls remained uncolored, and

seemed to be unaffected by the colloids; but when swelling occurred in the outer portions of the cell walls, as in a mixture of gold or platinum with NaOH, the swollen portions became deeply colored. In these cases the appearance of the colored wall was similar to that produced by the action of a soluble stain like Bismarck brown, a feature which suggests specific adsorption of the suspensoid as the cause of this color change. BREDIG (**2**) and ZSIGMONDY (**16**) mention similar instances of the staining of fungi by colloidal gold. In the former case this was not taken into the filaments but was deposited on the external surfaces of the walls. In the latter case the gold continued to be absorbed until the solution became entirely decolorized.

Apparently the only other published work on the effect of colloidal metals on plants other than bacteria is that of GALEOTTI (**6**). This author carefully studied the effect of colloidal solutions of copper on a species of *Spirogyra* and compared with these the effect of $CuSO_4$ solutions computed to contain the same amounts of copper in the ionic condition. GALEOTTI found colloidal copper poisonous at lower concentrations than was ionic copper, although the action of the former was less rapid. The author concluded that ionic copper produced a rapid effect by combining with the protoplasm, while the colloidal metal, at least in lower concentrations, was slowly active as a catalyser, accelerating certain breaking-down processes within the cells. Addition of 0.01 per cent of NaCl to higher concentrations of colloidal copper rendered these somewhat less toxic and almost entirely corrected the toxicity of the colloid in lower concentrations. The toxicity of ionic copper, on the other hand, was not modified in the presence of 0.01 per cent of NaCl. The influence of the chloride in diminishing the toxic effect of colloidal copper was attributed to some change induced in the colloid itself.

Following GALEOTTI, it may be suggested that the diminution in the toxicity of the lower concentrations of colloidal silver, brought about in the present studies by the inorganic salts of Crone's solution, may have been due to some action of the salts upon the colloid. The effect of animal charcoal and of colloidal platinum upon the toxicity of colloidal silver, that of colloidal platinum upon the

toxicity of toxic waters and salt solutions, and that of colloidal platinum, gold, and silver upon the toxicity of NaOH may be considered as probably due to surface phenomena such as adsorption. It appears from the experiments that ionic gold and ionic platinum ($AuCl_2$ and $PtCl_4$) exerted no influence upon the toxicity of NaOH and $MgSO_4$, respectively. The information at hand is not sufficient to warrant any suggestion as to why the transfer of filaments from colloidal gold solution to non-toxic distilled water was so quickly fatal to *Spirogyra*. A possibly similar case, in which the toxicity of a solution was decreased by addition of a colloid, has been reported by HATCHER (7), who found that the toxicity of strychnine injected hypodermically into frogs and guinea-pigs was considerably decreased when the alkaloid was administered in a solution of gum acacia. In this connection it needs only to be suggested that the finely divided and otherwise highly absorbent solids which, as has been shown by various authors since the time of Nägeli (NÄGELI 14, TRUE and OGLEVEE 15, BREAZEALE 1, LIVINGSTON *et al.* 11, LIVINGSTON 12, JENSEN 9, HOYT 8), exert a correcting influence upon many toxic solutions, partake of some of the properties of colloids. One essential feature, in this regard, of all such solids, as well as of both suspensoids and emulsoids which exert such corrective influence, seems to be great extension of surface.

It is interesting to notice that, although colloidal platinum produced improvement in ordinary distilled water, a weak solution of agar, when added to such water, produced no improvement. It has also been pointed out in another paper (8) that a 1 per cent agar hydrogel made with this water was found to be only slightly toxic, while a similar 2 per cent agar hydrogel was decidedly injurious. Evidence is not at hand for any attempt to interpret these phenomena critically.

The results obtained furnish no indication as to what physicochemical properties may be ascribed the extreme toxicity of colloidal silver and copper when colloidal gold and platinum are so slightly injurious. These results agree with those obtained by FOÀ and AGGAZZOTTI (5), who showed that, although colloidal platinum, gold, and silver were about equally effective in hindering the development of bacteria, colloidal platinum and gold in strengths of

125 ppm. were fatal to only two kinds of the bacteria tested, while finely divided colloidal silver containing 70 ppm. was fatal to all the bacteria used in their investigations. MORSE (13), from some preliminary experiments on several unicellular animals and plants, concluded that colloidal platinum and gutta percha are without appreciable effect on protoplasm. It should be noted that, in the present investigation, alga filaments in colloidal solutions often showed injury suddenly, after they had remained for several days in apparently excellent or good condition.

The swelling of cell walls caused by akaline colloidal gold or platinum, as observed in these studies, seemed to depend upon the presence of both the colloid and the alkali, since the walls seemed unaffected by the colloid alone and were very slightly altered by a solution of NaOH alone, while marked swelling was produced by a mixture of colloid and NaOH. A mixture of $AuCl_2$ and NaOH was without effect in this connection. The failure of colloidal silver to induce swelling may possibly be associated with those properties of this colloid that produced its toxic effect upon the protoplasm. That no sheaths were formed in those alkaline solutions of silver that permitted the alga to live in apparently good condition may be related to the extreme dilution of these silver solutions.

Results strikingly similar to those obtained in the present investigation from the use of colloidal gold and platinum with NaOH were obtained by KLEBS (10) upon precipitating various substances (as calcium phosphate, copper sulphide, iron ferrocyanide, alizarine-lead oxide) within the gelatinous sheaths of several species of *Zygnema* and other algae. Under these conditions there were formed colored, swollen, gelatinous masses, which separated from the cells in a way quite like that described above. This swelling occurred not only on the walls of living cells, but also on the walls of cells killed by ether vapor or weak alcohol, although not on the walls of those killed by corrosive sublimate, lead acetate, or some other substances. Not all precipitates caused swelling, and the appearance of the gelatinous masses, when formed, differed with different precipitates and different species of algae.

Since the formation of these swollen masses was caused only by precipitates composed of small particles, while it was caused by

widely different chemical substances, and furthermore, since substances deposited as crystals within the sheaths were without effect in this respect, KLEBS concluded that the swelling did not depend on the chemical nature of the precipitated substance, but did depend on the size of the particles composing the precipitate. He was led by these and other considerations to the view that the presence of solid particles between the particles of the gelatinous sheath was the mechanical cause of processes which ended in the breaking-off of the jelly along with the precipitate. He described this result as being brought about by two steps: first, the particles which were precipitated within the sheath slipped out, surrounded by portions of the jelly, and gathered at the periphery, where they were cemented together by the jelly into a layer surrounding each filament; second, this layer containing the precipitated particles was raised up in irregular forms and thrown off by the swelling of the jelly. He concluded that the swelling was not due to a reaction of the protoplasm to a stimulus, that substances which affected the protoplasm also affected the structure of the jelly and consequently its capacity for swelling, but that the observed phenomena were not then explainable in their molecular-physical relation.

On grounds of physical chemistry, as has already been suggested, it seems possible to bring such phenomena as those observed by KLEBS and those of the present studies into the same category with many cases of enzymatic catalysis. Disintegration of cell walls in plants, related to enzyme action, is frequently accompanied by such alterations in the material of the walls as to increase the extent of their imbibing power, so that they swell much more than is usual and assume a gelatinous or mucilaginous consistency. That some colloidal metals (as platinum) may exert influences upon organic material, that are quite similar in many respects to the effects of enzymes, is now generally held. Furthermore, the acidity or alkalinity of the medium employed is well known to influence greatly enzymatic catalysis, some enzymes being more effective in an acid and others in an alkaline solution. That enzymes are characteristically colloid and that their activity is closely related to this fact seems also to be established by the work of many biochemists. The possibility is suggested, therefore, that the disintegration effects

above described, produced in alkaline solutions of colloidal metals, may be considered as catalytic phenomena due to the colloids, the latter acting in a manner more or less similar to that in which the organic colloidal catalytes which we term enzymes are known to operate; if catalysis results frequently from enormously extended surfaces, it may not be without the bounds of probability to suppose that alterations in organic material, similar to those produced by the organic enzymes, may be brought about by inorganic colloids such as those dealt with in the present paper. It seems also possible to relate the swollen cell walls observed by KLEBS, in the study above discussed, to some catalytic action which has altered the water-imbibing power of the wall material, rather than directly to the forces which are active in producing absorption and formation of his precipitates. To attempt the extension of this general hypothesis to the details of the various instances would be out of place at the present time.

It is worthy of note that the swellings with which this paper has had to do were most pronounced in *Spirogyra decimina*, less so in *S. longata* from the stock culture, and were not exhibited at all by the latter form from the culture in Crone's solution. That the last mentioned material was the most vigorous, while the stock material of *S. longata* was next in order, and the material of *S. decimina* was apparently least healthy, suggests that the portion of the cell wall taking part in the swelling differed in the three sorts of filaments; susceptibility to the swelling influence exerted by the colloidal metals seems to have been greater as the alga was less vigorous. Whatever may have been its explanation, we have here another example illustrating the fact that similar filaments of the same algal species, but cultivated under different conditions, may be expected to attain different physiological states, thus becoming physiologically quite dissimilar. Such differences are apparently indicated in the present instance by the diversity above noted in the susceptibility of the outer portions of the cell walls in the three kinds of algal material. The existence of different physiological states in individuals of a single species is of course well known to all experimenters. It has been previously shown for *Spirogyra*, by the present writer (8), by parallel and exactly similar experiments

carried out with plants from the same original stock but kept for some time under different cultural conditions. In experiments designed to test the effect of special environmental conditions upon organisms it is absolutely necessary to guard, as far as may be possible, against these different physiological states in the material employed. Such a statement is self-evident, yet many results and criticisms found in physiological literature seem to have been brought into existence through failure to take this fundamental condition sufficiently into account.

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Summary

1. Colloidal silver was fatal to filaments of *Spirogyra* in all concentrations above 0.045 ppm. and was injurious in concentrations as low as 0.00225 ppm. The weaker solutions of silver were rendered almost or entirely non-toxic, during the period of the experiments, by addition of colloidal platinum, animal charcoal, or inorganic salts to form a 0.5 per cent Crone's solution.

2. A solution containing 90 ppm. of colloidal gold and approximately 0.02 per cent of NaOH was only very slightly injurious.

3. A solution containing 96 ppm. of colloidal platinum was almost non-injurious during the period of the experiments, and, in less concentrated solutions, partially corrected the toxicity of tap water, ordinary distilled water, and solutions of KCl, $MgSO_4$, and colloidal silver.

4. Colloidal gold, colloidal platinum, and, to a less extent, colloidal silver, in low concentration, all partially prevented injury to the alga filaments by toxic solutions of NaOH. Addition of $AuCl_2$ to a toxic solution of NaOH, or of $PtCl_4$ to a toxic solution of $MgSO_4$ did not render the hydrate solutions less toxic.

5. When *Spirogyra* was placed in a solution containing colloidal platinum or colloidal gold together with NaOH, the outer portions of the cell walls swelled, forming crumpled, gelatinous sheaths

which became deeply stained by the metal. This swelling was especially pronounced when the filaments were transferred from the alkaline colloidal gold or platinum solution to non-toxic distilled water. The swollen masses thus produced often parted from the rest of the wall, leaving the latter uncolored and apparently unaffected.

6. The cell walls were apparently unaffected by colloidal silver, either alone or with NaOH, or with salts to form a 0.5 per cent Crone's solution. They seemed to be unaffected by colloidal platinum alone or by a mixture of this with colloidal silver. Only very slight swelling of the walls occurred in solutions of NaOH alone. Marked swelling occurred only with the solution of colloidal gold or of colloidal platinum in the presence of NaOH. A solution of $AuCl_2$ and NaOH was without effect in this regard.

7. Filaments of *Spirogyra* originally from the same culture, but grown for a time in different media, exhibited different reactions in the solutions of colloidal gold and NaOH, as well as in the other toxic solutions here employed.

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THE FUNCTION OF MANGANESE IN PLANTS

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Historical introduction

Dating from the time of SCHEELE (1), numerous investigators have noted the presence of manganese in plants of various orders. While small amounts only of this element have been found in most plants, in 1860 HILGARD (2) pointed out that the ash of the long-leaf pine from Mississippi contains a relatively high percentage; and in 1878 J. SCHROEDER (3) found in the ash of the Norway spruce (*Picea excelsa*) 35.53 per cent Mn_3O_4, and in the ash of the bark 41.23 per cent. These and other observations, however, received but little attention for many years. Manganese occurs in small amounts in practically all soils, but being an element unessential to growth and normal development, its absorption by plants was considered to be without physiological significance.

The discovery of BERTRAND (4) in 1897 of the occurrence of manganese in the oxidizing enzymes of plants, however, and the subsequent finding that small amounts of manganese salts stimulate the oxygen-carrying power of these catalytic agents, have drawn attention to this question and have led to the view that after all a physiological rôle is probably played by this element. Since the time of these discoveries a large number of experiments, with a wide range of plants, have been made, various compounds of manganese in water and soil cultures being used; and, in general, it has been found that small amounts of manganese bring about stimulation in growth. While small amounts often produce stimulation, wherever more than a very low concentration has been employed, toxicity has resulted.

LOEW (5) and his co-workers in Japan found, furthermore, the toxic concentration in water cultures to be different for different species of plants. A concentration that was stimulating to rice, for instance, proved to be toxic to barley. Likewise, SALOMONE (6) observed in field experiments that the application of such small amounts as 40 kilograms per hectar of manganous sulphate pro-

duced injurious effects on wheat, while other investigators have found stimulation to result from the application of much larger amounts. In a number of instances, however, the application of different amounts of manganese in field culture has produced no apparent effects.

Regarding the specific effects produced by manganese, BERTRAND, as stated above, and a number of other investigators, have shown that, on the one hand, the addition of small amounts of soluble manganese increases the oxygen-carrying power of the oxidases, and on the other, stimulation is produced in the oxidizing power of infusions from plants grown under the influence of manganese compounds. During recent years an increasing importance has been attached to the oxidizing conditions of the soil, and, as has been pointed out in the researches of SULLIVAN and REID (7), there appears to be a direct correlation between the growth of plants (fertility of the soil) and the oxidations going on in the soil. The catalytic oxidations in soils have also been found to be proportional, within certain limits, to the percentage of manganese contained therein, and to be susceptible of stimulation by the addition of manganese compounds in much the same way as stimulation in the activity of plant oxidases is produced by the application of manganese salts.

Certain other investigators have also studied the effects on the solubility of plant nutrient constituents of soils produced by the application of manganese compounds. BERNARDINI (8), for example, has shown that manganous chlorid causes à mobilization of calcium and magnesium from both soils and mineral silicates to a greater degree than is produced by potassium, sodium, or ammonium chlorides.[1] From these facts the conclusion has been drawn that plant stimulation, resulting from the application of manganese compounds, is probably due to indirect effects on the inert bases of soils. As bearing on this phase of the question, ASO (9) also pointed out that while a 41.8 per cent increase in the yield of rice was obtained from the first application of manganous sulphate, similar applications to the same soil the following year produced only a 2.2 per cent increase.

[1] See NOTTIN, Ann. Sc. Agron. Ser 4. pp. 1–12. 1913.

The toxic effect above mentioned usually manifests itself by a dying-back of the growing tips and the yellowing of the leaves. LOEW and SAWA (5) hold that the activity of the oxidizing enzymes of plants may become excessive under the influence of sufficiently great amounts of manganese, resulting in the auto-oxidation of the chloroplastids, thus destroying the green pigment. SALOMONE (10), on the other hand, found evidences of plasmolysis in wheat that had been fertilized with manganese dioxide at the rate of fifty kilograms per hectar. BRENCHLEY (11) also observed a toxic action from manganous sulphate when it was applied in the very low concentration of one part per one hundred thousand parts of culture solutions. When it is remembered, however, that many plants vegetate normally in culture solutions varying rather widely in ionic-concentration, without showing evidences of plasmolysis, in other words, the ionic-concentration of the cell sap considerably exceeds that of ordinary culture solutions, it seems improbable that the addition of such small amounts of manganous sulphate would produce plasmolysis as a direct effect.

From the foregoing brief résumé[2] of the experiments on this question, it will be seen that two different sets of views have been held by which the action of manganese on soils and plants has been explained. These may be briefly stated as follows: (1) manganese stimulates the necessary oxidations going on in soils and plants through the activation of the oxidizing enzymes, etc.; (2) the application of soluble manganese brings about plant stimulation by rendering soluble, and therefore making available, the essential plant food of soils.

Under the first named of these theories may be included the stimulated oxidations, in both soils and plant tissues, the former probably being referable to the action on certain microorganisms, as well as on soil catalysis, while the latter has to do with the physiological processes within the cell. Viewed in the light of the last named theory, the effects of manganese on plants are considered to be indirect and due to its increasing the solubility of the mineral constituents of soils.

[2] A more complete bibliography of literature dealing with this subject will be found in a paper by the writer published as Bulletin 26, Hawaii Experiment Station.

A study of the literature on this subject, embracing as it does experiments with a considerable range of plants, reveals discrepancies, and, in the opinion of the writer, casts doubt on the adequacy of either one or both of the above mentioned conceptions as furnishing sufficient basis for a complete explanation of the function of manganese in plants. In this connection, the application of the principles of plant physiology, particularly osmosis in its bearing on the absorption of chemical substances from soils, naturally raises the question of the influence of soluble manganese on the selective absorption of the necessary nutrient elements. SCHREINER and SKINNER (12) have shown, for example, that small amounts of cumarin materially alter the absorption of potash by wheat seedlings; that quinone modifies the osmotic absorption of phosphoric acid; and that vanillin and dihydroxystearic acid exert a noteworthy influence upon the absorption of nitrates. Up to the present time the fundamental steps and the specifics of these reactions have not been elucidated. The exact "how" as yet can only be inferred. With regard to the specific effects on the osmotic phenomenon produced by small amounts of inorganic elements occurring in soils, other than the so-called plant food elements, little indeed is known. It is reasonable to suppose, a priori, that some osmotic effect would be produced. In fact, it seems improbable that the presence of any considerable amount of an electrolyte in the nutrient solution would exert a strictly neutral effect.

For a number of years the writer has studied the effect of manganese on plants. A preliminary report of this work was published in 1908 (13), in which it was shown that certain soils of Oahu, used for pineapples, contain abnormally high percentages of manganese. On these areas the pineapple undergoes the phenomenon of chlorosis, the leaves becoming yellow and the fruit pink in color throughout the entire period of its growth. The growth of the plant is stunted and the fruit produced is inferior in size. Further investigation of this question has led to a study of the function of manganese in plants in general, the results of which, in view of the prominence of the question, appear to be of sufficient interest to warrant a brief discussion at this time.

Experimental observations

The macroscopic appearance of a number of plants when grown on manganiferous soil is characteristic. Pineapples develop chlorosis at an early age, from which they never recover. The effects are first noticeable by a yellowing on the margins of the leaves or the development of yellow spots which soon spread over the entire leaf. The growing tips die back, the leaf margins becoming brown. Usually such plants produce small fruit of an abnormally pink color. Pineapple roots, instead of maintaining the usual pointed growing tip, are often found to be greatly enlarged, sometimes to the size of a lead pencil. The roots of other plants also become modified to some extent. Those of *Panicum molle* are found to be abnormally woody. Others are similarly affected. Corn makes poor growth, the leaves turning brown at an early stage and the stalks taking on a deep purple color. Barley, oats, and rice are likewise stunted in growth. Of the Leguminosae, the cowpea (*Vigna catjang*), in particular, is very sensitive to manganese; the lower leaves become brown, die back from the tips, and fall away. The pigeon pea (*Cajanus indicus*) is affected somewhat similarly. Onions also die back from the tips and produce very small bulbs.

Some species, however, appear not to be affected. The *Agave sisalana* shows no effects; sugar cane is not greatly affected, and cotton and tobacco, although somewhat retarded in growth, are not otherwise affected. *Waltheria americana*, the sow thistle, and a species of *Crotalaria* grow as weeds on the manganiferous soils and are in no way hindered in their development. These illustrations could be greatly enlarged, but are sufficient to indicate that the apparent effects of manganese in different species are far from being uniform.

Microscopical studies.—From a microscopical study of the several parts of these plants, it was found that in those instances where manganese exerts a toxic effect, the cell walls of the root cortex become brown, and in some instances contain minute granules of manganese dioxide. In a few instances the cells having brown cell walls extend into the central cylinder of the root, and

it is not uncommon to find a thickened tissue lying next to the cell walls in the angles of the cells. In the more advanced stages of chlorosis the protoplasmic contents of pineapple leaves become contracted into formless masses and appear to undergo partial decomposition. Normally, the palisade cells (14) of this species contain not more than mere traces of chlorophyll, being essentially water-storage tissue. Under the influence of manganese the liquid content becomes coagulated and draws away from the cell walls; likewise the protoplasm in the chlorophyll-bearing cells breaks away from the cell walls in places, contracting into an irregularly shaped mass. Plasmolysis, therefore, takes place, and in a few instances the nuclei become brown.

At the beginning of the chlorosis there is a fading of the green color without any other noticeable change; but in successive stages the chloroplastids gradually become fewer in number and smaller in size until finally they almost completely disappear. Simultaneously with the fading of the chlorophyll, a diminution in the starch contents takes place, and as the chlorosis develops, less and less starch is found adhering to the chloroplastids, until finally it entirely disappears. In the chlorotic plants the palisade cells also seem to be given over to the storage of calcium oxalate, and the occurrence of this metabolic by-product is greatly increased in practically all parts of such plants.

OXIDIZING ENZYMES.—The view that manganese exerts an influence on plants, through stimulating the auto-oxidations (so indispensable to living matter), gives interest to a study of the oxidizing enzymes occurring in plants from manganiferous soils. Accordingly, the oxidase and peroxidase activities of infusions obtained by crushing the leaves were measured empirically by use of the guiacum and aloin reactions. The results showed that while infusions from some chlorotic plants contain vigorous oxidizing enzymes, frequently such infusions give much weaker oxidase and peroxidase reactions than normal plants. These tests have been applied to infusions from all parts of pineapple plants, coming from soils containing manganese in amounts varying from 0.01 to 9.75 per cent, and with plants at all ages and in the various stages in the development of chlorosis; but when applied to a large series of

samples no correlation was found between the percentage of manganese in the soil or the degree of chlorosis on the one hand, and the activity of the oxidizing enzymes on the other.

These tests have also been made with various other plants, some of which show toxic effects from the manganese, and with still others that appear not to be affected. On the whole, the results again fail to show any relation between the activity of the oxidizing enzymes and the percentage of manganese in the soil, or the toxic effects.

As is well known, the activity of oxidizing enzymes in different specimens of a given species of plant is by no means uniform when grown on normal soils. It has been found, for instance, that extracts obtained from the fresh leaves of normal sugar cane and pineapples, respectively, varied in their oxygen-carrying power between wide extremes. It is also known that pathological disturbances, caused by attacks of Aphidae, the mosaic disease of tobacco, etc., are also associated with accelerated oxidation. The autumnal yellowing of plants, incident to maturity, and the development of yellow spots on certain plants, have likewise been shown by WOODS (15) to be associated with an increased activity of the oxidases and peroxidases.

It seems reasonable to suppose, therefore, that while manganese has the power of increasing the oxygen-carrying power of oxidases, and consequently may, in this way, to some degree bring about plant stimulation, the phenomenon of chlorosis cannot be completely explained on the basis of excessive auto-oxidation. The development of chlorosis under the influence of manganese is very probably the result of physiological disturbances of a more deep-seated nature.

COMPOSITION OF THE ASH.—A number of ash analyses have been made for the purpose of determining the effects of manganese on the absolute, as well as the relative, absorption of plant food elements. The materials for analysis were selected with the greatest care, so as to secure representative plants of the same age and stage of development in a given species. The results are recorded in table I.

The data given in table I bring out some interesting facts. In

TABLE I

ASH ANALYSIS OF PLANTS GROWN ON NORMAL AND MANGANIFEROUS SOILS

Plants analyzed	Manganese (Mn_3O_4)	Lime (CaO)	Magnesia (MgO)	Phosphoric acid (P_2O_5)	Total ash
Pineapple leaves 5 months old:	*P ct.*	*P ct.*	*P ct.*	*P ct.*	*P ct.*
Manganiferous soil	2.41	9.01	5.70	2.81	9.94
Normal soil	1.70	7.14	7.60	3.57	7.14
Pineapple leaves 18 months old:					
Manganiferous soil	2.08	15.66	7.91	1.66	7.98
Normal soil	1.40	7.00	6.98	2.70	6.24
Pineapple stalk 5 months old:					
Manganiferous soil	.25	36.42	2.60	6.12	8.85
Normal soil	.25	23.87	5.82	8.86	5.12
Pineapple stalk 2 years old:					
Manganiferous soil	.80	14.36	7.75	6.70	7.78
Normal soil	.20	12.96	5.78	8.36	6.60
Corn stover:					
Manganiferous soil	.40	8.60	7.64	5.17	7.55
Normal soil	.15	3.45	4.60	7.56	9.18
Cowpea vines:					
Manganiferous soil	3.07	22.72	6.93	2.90	13.73
Normal soil	.87	17.10	9.13	5.03	13.44
Cowpeas:					
Manganiferous soil	.15	1.47	4.15	15.89	3.93
Normal soil	.00	1.13	6.91	22.71	3.53
Paspalum orbiculare:					
Manganiferous soil	2.20	5.30	3.46	2.54	7.88
Normal soil	1.05	6.70	5.05	2.34	7.16
Guava leaves:					
Manganiferous soil	.82	43.02	3.73	4.47	7.66
Normal soil	.28	22.98	10.46	7.70	6.73
Guava wood:					
Manganiferous soil	1.20	52.10	1.51	4.33	5.87
Normal soil	.15	26.17	7.22	6.97	4.02
Sugar-cane leaves:					
Manganiferous soil	.45	14.33	4.41	3.21	5.57
Normal soil	.60	16.60	6.07	4.03	4.85
Crotalaria:					
Manganiferous soil	1.40	30.80	7.79	4.12	6.77
Normal soil	.05	19.00	21.05	9.13	6.28
Peanut leaves:					
Manganiferous soil	.25	35.24	5.61	4.49	10.45
Normal soil	.04	39.41	10.99	4.97	9.95
Peanut stems:					
Manganiferous soil	2.56	14.80	9.04	2.00	11.15
Normal soil	.32	21.76	19.02	4.81	7.12
Ironwood (*Casuarina equisetifolia*) needles:					
Manganiferous soil	.66	37.50	4.57	1.62	.....
Normal soil	T.	43.12	7.75	4.23	.....
Olive leaves:					
Manganiferous soil	.64	26.32	1.84	2.63	.....
Normal soil	.86	17.80	1.53	2.78	.....
Waltheria americana leaves:					
Manganiferous soil	8.70	31.30	3.81	2.80	.....
Normal soil	.82	29.62	5.44	3.81	.....

TABLE I—*Continued*

Plants analyzed	Manganese (Mn_3O_4)	Lime (CaO)	Magnesia (MgO)	Phosphoric acid (P_2O_5)	Total ash
Waltheria americana stems:					
Manganiferous soil	.56	14.97	11.70	3.67	3.58
Normal soil	.45	13.70	11.30	3.46	3.73
Broom-corn leaves:					
Manganiferous soil	2.24	12.08	6.75	.94	.....
Normal soil	.60	5.44	3.52	1.38	.....
Broom-corn stalks:					
Manganiferous soil	1.36	2.12	3.12	1.02	.....
Normal soil	.52	1.88	2.51	3.72	.....
Tobacco stems:					
Manganiferous soil	.57	9.47	4.08	2.08	7.02
Normal soil	T.	1.88	3.27	7.35	8.50
Pigeon-pea leaves:					
Manganiferous soil	1.43	16.11	3.25	4.04	12.17
Normal soil	.15	7.86	7.66	10.85	7.84
Pigeon-pea stems:					
Manganiferous soil	.99	15.29	2.82	4.25	5.81
Normal soil	T.	3.79	4.39	9.34	6.22
Oat straw:					
Manganiferous soil	.86	9.15	5.16	.73	10.27
Normal soil	T.	3.40	3.19	8.81	11.73
Wheat straw:					
Manganiferous soil	.22	4.51	4.03	2.96	8.91
Normal soil	T.	2.09	2.74	5.56	16.27
Mango leaves:					
Manganiferous soil	2.16	33.12	2.15	2.89	9.24
Normal soil	.24	17.13	4.90	5.43	8.21

practically every instance the absorption of manganese was increased on the manganese soil. The analyses further show that there is a pronounced difference in the percentages of lime, magnesia, and phosphoric acid in the ash of plants from the two classes of soils. Some of the plants were not visibly affected; others little so; while still others showed a pronounced toxic effect; but uniformly throughout there was a tendency toward an increased absorption of lime on the one hand, and a decreased absorption of magnesia and phosphoric acid on the other.

Under certain conditions the ratio of lime to magnesia seems to be of considerable importance to plants. In order to bring out more clearly the relations between these two constituents of the ash, the relative amounts of lime and magnesia absorbed from normal and manganiferous soils, respectively, have been recalculated from the previous ash analyses and are presented in table II.

An inspection of the data in table II shows that in almost every

TABLE II

THE RATIO OF LIME TO MAGNESIA IN PLANTS (MAGNESIA CONSIDERED AS 1)

Kind of plant	From manganiferous soil	From normal soil	Kind of plant	From manganiferous soil	From normal soil
Pineapple leaves:			Ironwood needles....	8.20	5.56
5 months old.....	1.58	.94	Olive leaves.....	14.30	11.63
18 months old.....	1.98	1.00	Waltheria americana:		
Pineapple stalk:			Leaves.....	8.21	5.44
5 months old.....	14.00	4.10	Stems.....	1.28	1.21
18 months old.....	1.88	2.24	Broom corn:		
Corn stover.....	1.12	.75	Leaves.....	1.79	1.54
Cowpeas:			Stems.....	.67	.74
Vines.....	3.28	1.86	Tobacco stems.....	2.32	.57
Seed.....	.35	.16	Pigeon peas:		
Paspalum orbiculare..	1.53	1.32	Leaves.....	4.69	1.02
Guava:			Stems.....	5.42	.86
Leaves	11.53	2.19	Oat straw.....	1.79	1.07
Stems.....	34.50	3.62	Wheat straw.....	1.12	.76
Sugar cane leaves	3.25	2.73	Mango leaves.....	15.40	3.49
Crotalaria	3.95	.90			
Peanut:					
Leaves.....	6.28	3.58			
Stems	1.63	1.14			

instance the ratio of the absorbed lime to absorbed magnesia was increased under the influence of manganese.

Discussion

In this investigation it has been shown that different plants, when grown on manganiferous soils, are affected differently. Some species are stunted in growth and die back from the tips of the leaves, which turn yellow or brown, and sometimes fall off, and a general unhealthy appearance results. Other species appear to be unaffected, and, so far as can be judged, vegetate normally in the presence of manganese. Microscopic investigations show that in certain instances the protoplasm undergoes changes. Occasionally it draws away from the cell walls and the nuclei become brown. There is a manifest change in the protoplasmic contents of the roots.

The chlorophyll in a number of plants is affected; in pineapples it undergoes decomposition. Simultaneous with the destruction of chlorophyll, starch formation ceases.

The activity of the oxidizing enzymes in plants is herein shown to bear no relation to the destruction of chlorophyll under the

influence of excessive manganese. While the oxidases generally contain manganese as a normal constituent, or at least manganese is closely associated with the oxidases, and at the same time their oxygen-carrying power is accelerated by the presence of manganese salts, the foregoing investigations show that there is no correlation between the phenomenon of chlorosis in pineapples and the activity of the oxidizing enzymes. The decomposition of chlorophyll in this case, therefore, is not due to excessive autooxidation. This does not imply, however, that accelerated autooxidation in plants is without effect.

From the ash analyses it was found that manganese was absorbed in considerable quantities, and in nearly every instance was greater in the plants from manganiferous soil. These analyses also show that a disturbance in the mineral balance takes place. The percentage of lime is increased, while the percentage of magnesia and phosphoric acid is decreased. Some of the plants analyzed showed a markedly toxic effect from the manganese, while others appeared to be unaffected; but in practically every instance a modification of the mineral balance was observed, and this was found to follow the same direction in all species. The ratio of absorbed lime to the absorbed magnesia was increased under the influence of manganese, regardless of whether the plant showed a toxic effect or not.

It is claimed by LOEW (16) and others that various plants are affected differently by different ratios of lime to magnesia; certain species of plants vegetate most advantageously when the ratio is one to one; whereas still other species grow best when the ratio is one to three, etc. In the publications dealing with this subject, however, mention is usually made of the effects produced upon the appearance of plants, while but few ash analyses have been made in this connection. From the data at hand, however, it appears that where the physiological balance between lime and magnesia is disturbed, a corresponding influence is brought about in the composition of the ash.

Under the influence of manganese, plants automatically modify themselves in regard to the absorption of these two elements, and, as is believed, it is not so much the absolute amount of calcium and

magnesium in a given soil as the ratio between these two substances in solution in the soil moisture that determines the physiological effects. In the case of plants grown on manganiferous soil, the relative amounts of lime and magnesia actually absorbed become greatly different from those absorbed from normal soils, regardless of the amounts of these elements present in the soil.

From these evidences, we may believe, then, that an important effect of manganese is of an indirect nature, being due to its bringing about a modification in the osmotic absorption of lime and magnesia, and that the toxic effects are chiefly brought about by this modification, rather than as a direct effect of the manganese itself. As has been mentioned already, not all species of plants are equally sensitive to modifications in the lime-magnesia ratio; and from some recent experiments of GILE (17) it seems that the effects of a modification in this ratio are, to some extent, dependent upon the concentration of the culture solution. Likewise, different ratios are best suited to different species. Therefore, the effect of manganese may be very different on different soils and with different species of plants. With certain plants it is toxic on certain soils for the reason that the absorbed lime and magnesia are thrown out of their optimum ratio for this plant, while in others it may exert a stimulating effect by bringing this ratio more nearly to its optimum.

The small amounts of manganese in natural soils, therefore, probably perform a twofold function in plant growth: (1) it acts catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in plants; and (2) it tends to modify the absorption of lime and magnesia, perhaps by partially replacing calcium from insoluble combinations, but especially, through a direct effect on the osmotic absorption of lime and magnesia, increasing the former and decreasing the latter.

The absorption of phosphoric acid is likewise decreased in the presence of manganese. By reference to the preceding table of ash analyses it will be seen that frequently the ash of a given species of plant from manganese soil was found to contain not more than one-half as much phosphoric acid as from normal soil. The interference with the absorption of phosphoric acid would also tend to

bring about stunted growth and might be sufficient to account for the difference in size of the plants from the two classes of soil. Studies on the solubility of these soils have shown the manganese to be slightly soluble in water, but markedly so in dilute organic acids. Phosphoric acid coming into solution in the soil moisture would tend to be precipitated by the manganese as manganese phosphate, a compound, which can be dissolved only with difficulty, and therefore the absorption of phosphoric acid would thus be hindered.

Reference has already been made to the fact that certain organic substances exert appreciable influence on the rates of absorption of certain of the necessary elements. Here it is shown that a similar action is to be ascribed to manganese. How is this fact to be explained?

During recent years the protective action of salts shown to obtain in animal experiments by LOEB (18) has been found to apply also to plants.

OSTERHOUT (19) has shown from water cultures, for instance, that some of the essential elements may be toxic unless properly balanced by definite concentrations of certain other necessary elements. In other words, some at least of the essential inorganic elements seem to play both a nutritive and a protective rôle. Quite recently OSTERHOUT (20) has been able to throw considerable light on the mechanics of the protective action. By the use of an ingenious device he determined the electrical conductivity of living protoplasm, both before and after it was placed in solutions of sodium and calcium chlorides, etc. The results show that the electrical resistance of the protoplasm becomes greatly reduced upon standing in sodium chloride solution, thus indicating that sodium had been absorbed. But when the protoplasm was placed in a solution containing sodium and calcium chlorides, of the same ionic concentration, no such lowering of the resistance took place. Calcium hinders, then, the passage of sodium through living protoplasm; and therefore must be looked upon as lowering the permeability. OSTERHOUT has further shown this phenomenon to be reversible, and therefore the presence of one salt may actually cause an increase in the permeability to others. In certain instances

he found the protoplasm to undergo visible changes, which, however, were not necessarily injurious to it. Protoplasm, being of a colloidal nature, then, becomes physico-chemically altered when placed in certain solutions; and such alterations, in turn, affect its permeability.

By the application of the above named conceptions, we may infer that when plants are grown on manganese soils, the soluble manganese, coming into contact with the root hairs, is at first absorbed, up to a certain point, forming combinations with the protoplasm. Once these combinations are established, the permeability of the protoplasm becomes altered, whereby the absorption of lime is facilitated, while that of magnesium is hindered. Manganese, then, may be looked upon as forming a combination with the protoplasm which materially alters the relative absorption of lime and magnesia. Not all plants are equally affected by a variation from the normal ratio of lime to magnesia, and therefore under field conditions toxic effects may be produced in some plants, stimulation in others, while with still others the effects may be neutral.

The experimental facts presented in this paper and the interpretations made in no way negative the work previously done on this subject. The failure to observe a more active catalytic oxidizing reaction in plants from highly manganiferous soil does not argue that manganese is incapable of stimulating the activity of oxidizing enzymes. The normal soils of Oahu, from which plants were taken for comparison, contain small amounts of manganese, which, moreover, was found to be absorbed to a considerable extent by all the plants studied. It is probable, therefore, that the absorption of manganese from the normal soils of the Islands is sufficient to produce maximum stimulation in oxidase activity.

HAWAII EXPERIMENT STATION
HONOLULU

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THE DEVELOPMENT OF THE PROTHALLIUM OF CAMPTOSORUS RHIZOPHYLLUS

F. L. PICKETT

(WITH PLATES XII AND XIII AND EIGHT TEXT FIGURES)

In this region the spores of *Camptosorus rhizophyllus* mature from June to October. The long axis measures 18–24 μ, and the shorter 12–20 μ. They contain a few small oil globules, but are destitute of chlorophyll. The nucleus is inconspicuous in the living spore. The perinium is thick, dark, and shows many sharp, irregular ridges with areas of unequal thickness intervening (figs. 47–55). The exospore is seen with difficulty, if at all, as the perinium is very rarely pushed off when the spore germinates.

The spores germinate slowly. The greater part of the writer's material was taken from fronds collected October 26, 1912, and kept between sheets of filter paper in a book in the laboratory. On November 22 the sporangia, after being scraped from the fronds, and crushed lightly to free the spores, were sown on well sterilized soil in clay saucers. The saucers of soil were protected from currents of air by bell-jars supported on small blocks of wood to allow proper ventilation, and were kept moist in a well lighted greenhouse at an average temperature a little above 70° F. The first sign of green was noted with a magnifier on December 17. On February 4, 1913, abundant antheridia were found on the larger prothallia, and one week later a few archegonia were found. On March 22 many old antheridia and archegonia could be found on the large prothallia. Other spores collected October 26, 1912, were sown December 23, and kept under the same conditions as the above, but out of direct sunlight. In these cultures the first sign of green was noted January 30, 1913. Other spores sown January 3 and kept in the light, where on days of bright sunshine the temperature reached 80° F. at midday, showed the first sign of green on January 30. In all these instances the prothallia were composed of 3–10 cells before their color was noted on the soil. Spores sown on boiled tap water, and kept under the same conditions

as the above mentioned cultures, showed the beginning of germination in 10–14 days. While spores collected in October germinated best before the first of February, a considerable percentage germinated as late as the last of March, having been kept in the dry air of the laboratory in the meantime. Spores collected from the usual habitat in March show a higher percentage of germination than those collected earlier and kept in the laboratory. Only a few mature; unopened sporangia may be found in the field as late as March, so but few spores can be secured at that time.

All the spores sown at one time and under the same conditions do not germinate and develop uniformly into prothallia. A small bit of soil, bearing prothallia, taken from any one of the cultures mentioned above, would show, up to the first of May, widely different stages of prothallial growth. Many times the writer has found on the same bit of soil the intermediate stages from prothallia composed of two or three cells to mature plants 2–3 mm. wide, and bearing antheridia and archegonia. That some of the small prothallia were dwarf male plants is beyond doubt, because of the presence of antheridia. But that some of the spores were late in germination is clearly indicated by the constant recurrence of these gradations in development accompanied by a continual increase in the number of mature specimens. The plants from which the photographs reproduced in pl. XIII were made were taken at one time from the same portion of one culture, and had exactly the same conditions for germination and growth.

Germination of the spore

When the spores are placed under the proper conditions for germination, they absorb water and increase in diameter from one-fourth to one-half. The swelling is followed by a rupture of the perinium and the exposure of the spore contents. The writer has been unable as yet to determine whether or not there are two distinct walls within the perinium. If present, they are quite transparent. Through the opening in the perinium may be seen the oil globules, sometimes an inconspicuous nucleus, and after a few hours a few small chloroplasts. From this point two lines of development have been noted.

In the first case, as in the typical Leptosporangiatae, a small papilla is formed and cut off from the body cell by a wall. This papilla elongates to form the first rhizoid. It contains no chloroplasts. Occasionally two such papillae are formed before the division of the prothallial cell (figs. 54, 55). Following the formation of the first rhizoid, the body cell increases in size, the oil globules disappear, and the chloroplasts increase in size and number. The second division of the spore produces a new prothallial cell containing chloroplasts.

In the second case, the first division may produce two prothallial cells similar in size, and both containing chloroplasts. The basal cell of such a group may later produce a rhizoid. From the examination of many germinating spores and young prothallia, it seems quite as common for the spore to undergo one or two divisions before the formation of a rhizoid as for the rhizoid to result from the first cell division. Numerous specimens similar to fig. 52, but without a rhizoid, have been seen. As germination proceeds, the perinium is distended but is not cast off; it may usually be found attached to the oldest cell of mature prothallia (figs. 14, 15).

As has been stated, immediately after the rupture of the walls, light green plastids appear in the exposed portion of the cell (figs. 51, 54). With the further growth of the spore and the formation of new cells, these chloroplasts become larger and increase rapidly in number. Throughout the life of the prothallium the chloroplasts retain a distinct yellow tinge, imparting a characteristic color to the plant. They at all times show a quick reaction to intense light, crowding close to the vertical cell walls after a few minutes of brilliant illumination. The oil globules persist for a short time only, and may not be seen after the first division of the prothallial cell.

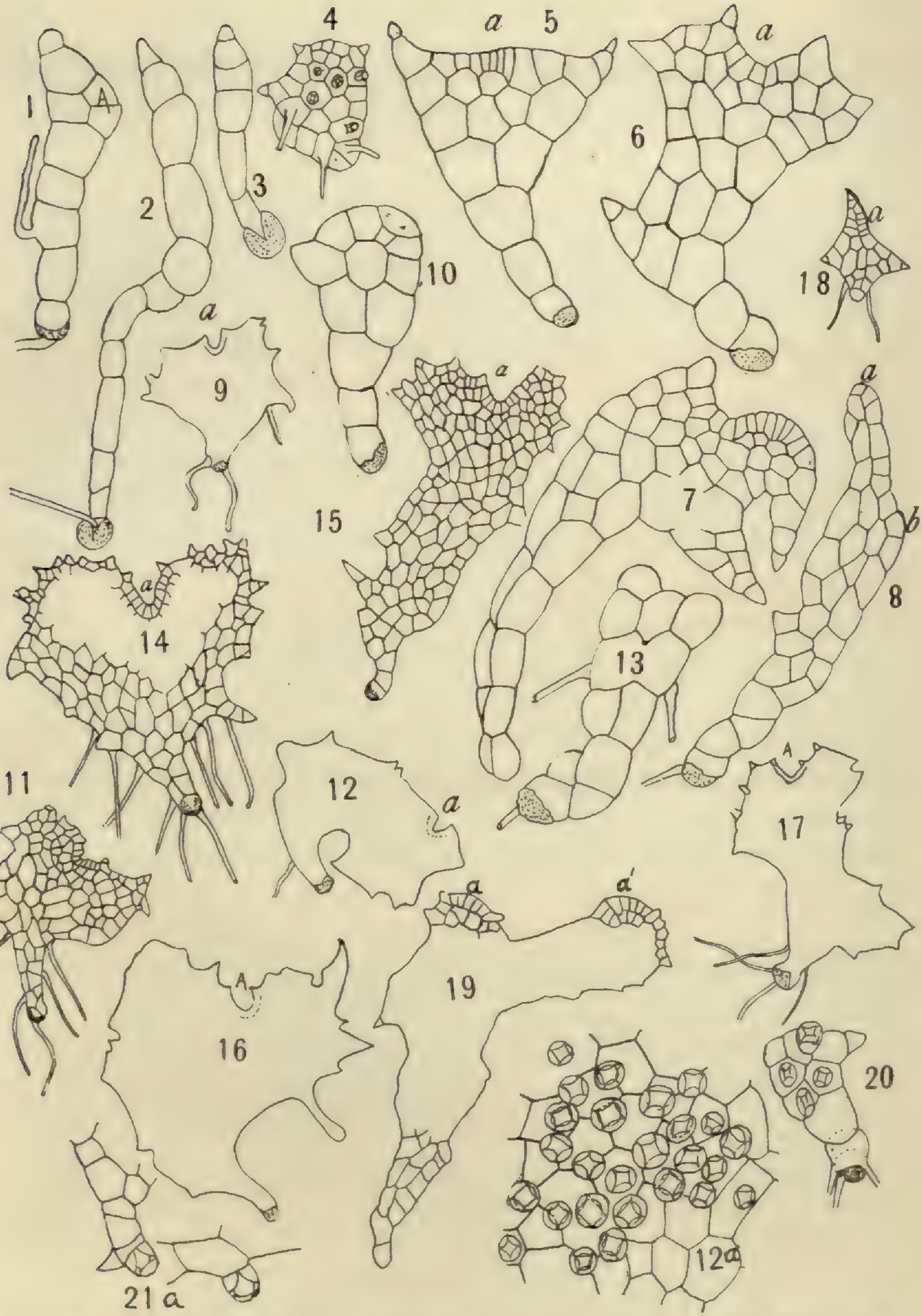
Early development of the prothallia

As was stated above, the first division of the cell may produce two similar cells, or may cut off a small cell which develops into a rhizoid. If the first division produces a rhizoid, the second division produces two cells similar in form and containing chloroplasts. Later divisions of the distal cell may occur in either a transverse

or longitudinal plane. In some instances consecutive transverse divisions occur until a long, protonema-like filament of many cells is formed (figs. 1, 2, 3, 61). The length of the filament may vary, but whether it be one cell or more than one cell in length, it finally produces, by longitudinal and further transverse divisions of its newer cells, a flat plate one cell in thickness. It should be noted that it is the rule for this plate to be formed by regular promiscuous divisions, without any suggestion of an apical cell or group, although exceptions may be found in unusually long filaments. Not infrequently this plate is formed immediately after the first transverse division of the spore (figs. 4, 18). In other specimens the plate formation begins after a chain of two or more cells is evident (figs. 5, 6, 8, 10, 61, 63). Occasionally a prothallium shows that transverse divisions have been followed or accompanied by longitudinal divisions until a strand two cells wide and as long as the simple protonemal structure mentioned above has been formed (figs. 7, 11, 14). Fig. 13 shows an intermediate form where one cell of a single row has given rise to two by longitudinal division. More rarely a definite strand three cells in width is clearly shown (fig. 19). That these strands, one, two, or three cells in width, are not a part of the regular prothallial plate is indicated by the abrupt beginning of the latter (figs. 5, 6, 7, 11, 14).

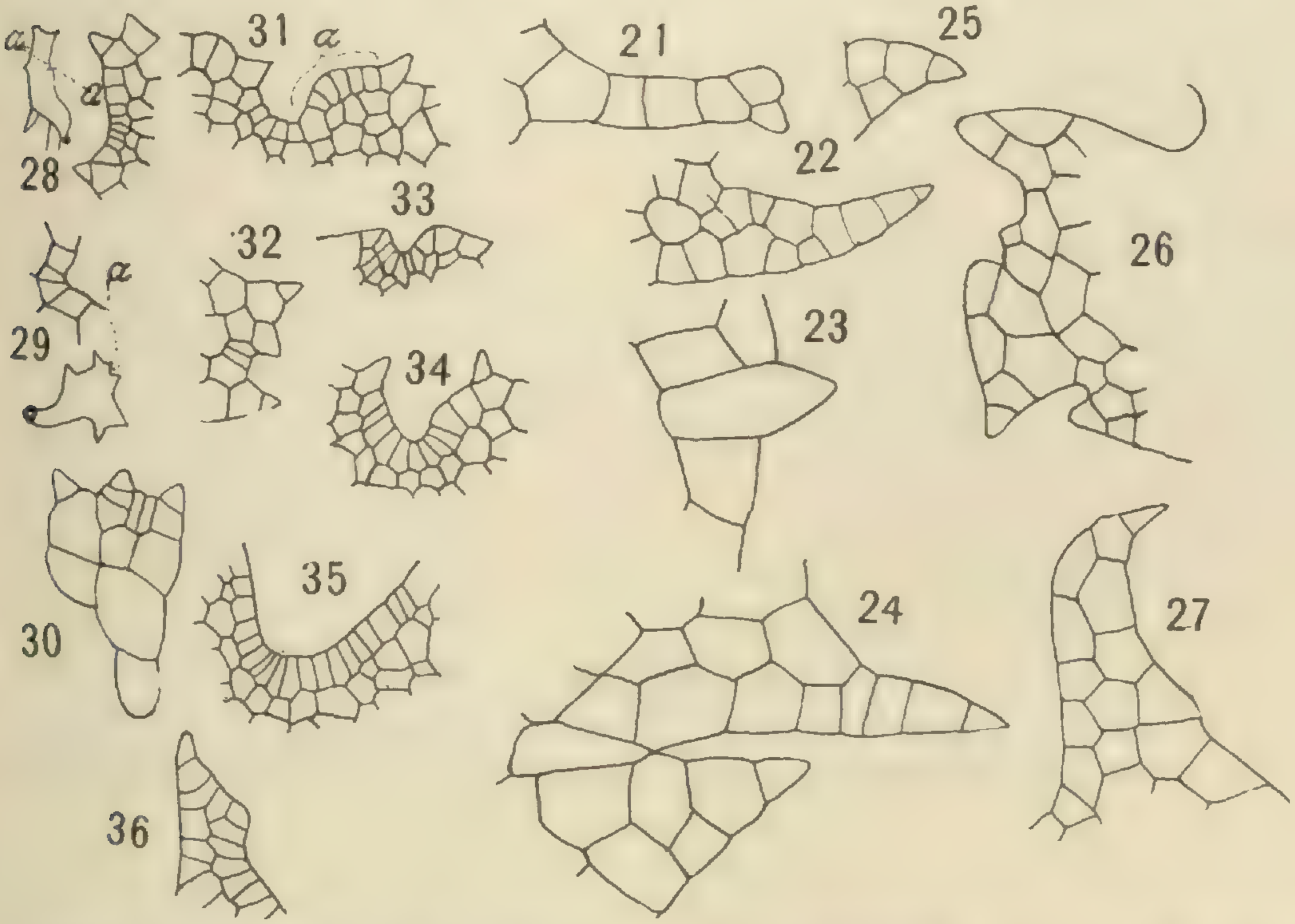
Later growth of the prothallia

The small prothallia increase in size by a promiscuous division of their cells in two planes. The location of growing regions and the direction of division of individual cells seem to follow no general rule. The resulting cell plates lack to a marked degree the regularity and symmetry usually found in the prothallia of related ferns. A glance at figs. 1–20 and at pl. XIII will give an idea of the many and various forms found. This continued promiscuous growth and division of the cells in the body of the prothallium is characteristic of *Camptosorus rhizophyllus*. In many cases prothallia of considerable size are formed by this growth alone before the formation of an apical region, as must have been true in those shown in figs. 15, 19, and 59. This type of growth continues after the appearance



FIGS. 1–21 *a*.—Figs. 1–3, protonema-like prothallia, with possible beginning of apical growth at *A* in fig. 1, ×120; fig. 4, small prothallium with mature antheridia, ×60; figs. 5 and 14, unusually symmetrical growth with distinct apical groups; figs. 6, 7, 8, and 10, examples of unsymmetrical forms, ×120; fig. 13, longitudinal division of one protonemal cell, ×120; fig. 15, prothallium with large plate of cells formed before appearance of apical group, ×40; fig. 19, prothallium with two growing points, ×40; fig. 20, very small prothallium with mature antheridia, ×120; fig. 21*a*, antheridia borne on marginal outgrowths, ×120; fig. 12*a*, group of antheridia from central part of fig. 12, ×120; the other figures show various unsymmetrical arrangements of apical groups.

of the apical region and even after the formation of the archegonial cushion or meristem. It is shown especially in the formation of various marginal outgrowths and in the plicate or crispate form of old prothallia. Such marginal structures, resulting from irregular



FIGS. 21–36.—Figs. 21–27, various marginal outgrowths; fig. 23, one-celled form growing directly from regular margin; see fig. 21*a* for outgrowths bearing antheridia; figs. 28–36, various forms of apical cells and groups; fig. 36 is *a* of fig. 18 more highly magnified; fig. 28, wholly lateral apical group, and fig. 31, apical group (*a*) beside a sinus; all ×120 except small figures in figs. 28 and 29.

cell multiplication having no connection with apical growth, are also characteristic of *Camptosorus*. These marginal structures may be of one or of very few cells (figs. 6, 17, 56, 61), or may be of many cells and approach the dignity of lobes (figs. 7, 16, 58). The form of several such lobes is shown in figs. 21–27.

The region of apical growth

Although the prothallia may attain considerable size and even mature gametes without the appearance of a typical V-form apical cell or a distinct apical group, such a growing region is quite usual and typical. The appearance of the apical cell or group is

varied as to both time and position. In long, protonema-like structures the beginning of a specialized growth region is usually indicated by the division of a cell once or twice removed from the distal end (fig. 1). In case a plate of cells has been formed as mentioned above, a group of cells anywhere about the periphery of this plate may become more active than the neighboring cells, and thus constitute an apical region or group. The formation of a distinct, individual, V-shaped apical cell has not been noted, although forms like figs. 8, 29, 30, and 32 suggest such. In the plant shown in fig. 8 the growing region would probably appear between *a* and *b* rather than as a result of any activity of the cell at *a*. In many instances the apical region is quite symmetrically placed (fig. 14); but an examination of figs. 7, 12, and 17 will show the extreme variation of position, and how far from symmetrical the placing of an apical group may be. Fig. 19 shows an even more interesting case, of which a few have been found, where two such regions have been

FIG. 37.—Large prothallium with distal margin almost plane as a result of activity of large apical group; ×28 and 160.

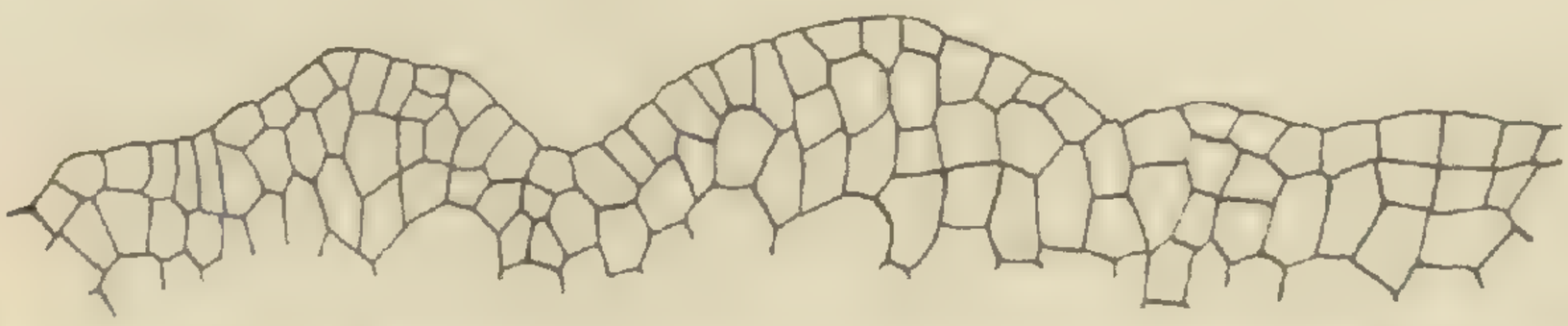


FIG. 38.—Portion of margin of large prothallium, showing almost continuous growing group; ×160.

formed. The very striking irregularity in the formation of the apical group is further shown in figs. 28–36 and in figs. 56 and 57. Fig. 28 with the group (*a*) entirely lateral to the evident axis of elongation, fig. 36 (an enlargement of *a* in fig. 18), and fig. 31 with the apical group beside a sinus are worthy of notice. Figs. 33, 34,

and 35 show about as regular or symmetrical a formation as has been found.

Another point to be noted is that as the prothallia grow older the apical group increases its activity, with the result that instead of being in a sinus of considerable depth it is pushed outward to form an almost straight margin at that place (fig. 37). Closely related to this phenomenon are the cases of proliferation now to be mentioned.

FIG. 39.—Old prothallium that has developed two special growing regions; ×28 and 160.

Prothallia which have grown for four or five months, and those which have been allowed to become quite dry and recover, sometimes continue irregular growth at one or more points on the margin. Fig. 38 shows a part of the margin of a large prothallium with renewed growth, after desiccation for several days. Fig. 39 shows two such regions on one prothallium. Fig. 40 shows an advanced development of such a region that has produced a rhizoid and might continue growth independent of the original prothallium. On some such proliferations antheridia are formed regularly and abundantly. The presence of archegonia has not yet been noted on them.

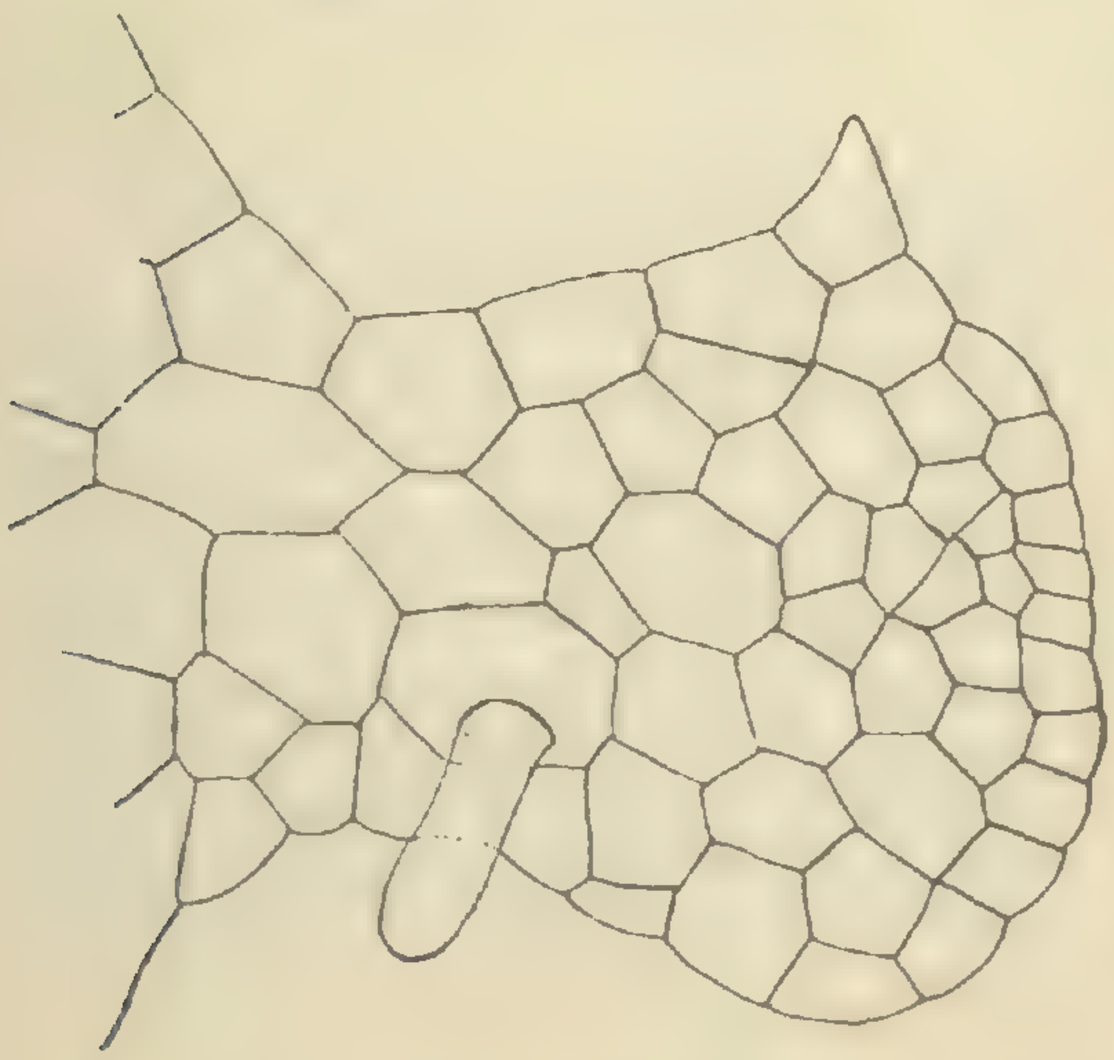


FIG. 40.—Special growing point similar to those in fig. 39, but more fully developed and showing rhizoid; ×160.

Size of prothallia

Prothallia bearing antheridia vary much in size at the time of maturing their first sperms. Examples of plants composed of but few cells and with mature antheridia (figs. 4, 20) are not uncommon; on the other hand, many reach a width of 1–2 mm. before producing their first antheridia. The dwarf antheridial plants never attain any great size; they are but one cell in thickness, and never produce archegonia. Fig. 41 shows the prothallium in fig. 20 and another taken at the same time from the same part of a culture and drawn to the same scale. These were just maturing their first antheridia. Antheridia are quite evenly distributed over the lower surface of the older portions of the large prothallia, and over the lower surface and margins of the dwarf forms (figs. 4, 12*a*, 20). Fig. 12*a* shows the abundance of antheridia on the large plant in fig. 12. Occasionally antheridia appear on the margins and even on the marginal outgrowths of large prothallia (fig. 21*a*). Five months old prothallia, bearing both antheridia and archegonia, are sometimes 4 mm. wide. They are strictly dorsiventral, although they show a marked tendency to take an upright position. The margins are distinctly crisped and plicate. After six to eight months, the marginal growth of the plants continues and the older portions die away much as in the liverworts.

FIG. 41.—Two prothallia of same age (as indicated by maturity of first antheridia) and from same bit of soil; ×28.

The rhizoids develop regularly from the lower surface of the prothallia, and are most abundant on the central part of the older portions, that is, at the base of the plant. They are long, slender, and sparingly branched near their free ends. The photographs reproduced in pl. XIII give a general idea of their character. The only point worthy of note is that mentioned above in connection

with the germination of the spore, namely, that the first division of the cell may produce a second prothallial cell instead of a rhizoid.

Antheridia and archegonia

The antheridia develop in much the same general way as described by CAMPBELL[1] for *Onoclea Struthiopteris* as a type of the Leptosporangiatae. The stages in development are suggested in figs. 42–46. The one point worthy of mention is the formation of the neck cell. This cell is cut off by a division of the initial antheridial cell in a plane parallel to the prothallial surface, and is very regularly found, although sometimes the more orthodox development shown in fig. 44 is evident.

The archegonial meristem is but little in evidence, being of small area and not conspicuous. It develops on prothallia soon after the appearance of the antheridia, and is found only on such plants as have a well formed apical group. Archegonia are found on plants 1.5 mm. or more in width. Their development is in every way typical of the Leptosporangiatae, but only a few (4–8) are found on a prothallium.

Summary

The spores of *Camptosorus rhizophyllus* germinate very irregularly in point of time.

Prothallia bearing antheridia only and those bearing both antheridia and archegonia are produced.

Both antheridial and archegonial prothallia show a wide variation in size and form, the result of a promiscuous cell division and growth.

The formation of a typical V-shaped apical cell is rarely found, if at all, and the apical group is usually unsymmetrically placed.

Old prothallia, bearing both antheridia and archegonia, may develop several marginal growing regions, and may even produce proliferations capable of independent growth.

The archegonia follow the typical Leptosporangiatae in their

[1] Mosses and ferns, 1905, p. 315.

development. The antheridia usually form a neck cell before the regular antheridial divisions.

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EXPLANATION OF PLATES XII AND XIII

(Figs. 1–41 in text)

PLATE XII

All figures ×660

FIGS. 42–46.—Stages in development of antheridia; fig. 44 shows orthodox form sometimes found, the others show more usual form with a neck cell.

FIG. 47.—Fresh, mature spore.

FIG. 48.—Spore with cell showing through ruptured coats.

FIG. 49.—Spore with rhizoid showing first.

FIGS. 50, 51.—Rhizoids issuing from the cell exposed by the rupture of the coats: *og*, oil globules.

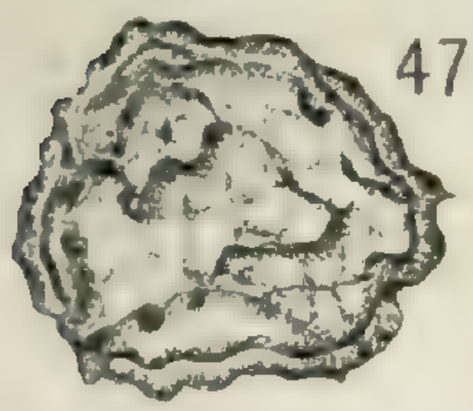
FIGS. 52, 53.—First and second divisions of the prothallial cell.

FIGS. 54, 55.—Two rhizoids formed by the undivided spore.

PLATE XIII

All figures ×75

FIGS. 56–64.—Photographs of living prothallia which, with many others, were taken at one time from one small bit of soil; only enough are shown to indicate the variation in form and growth.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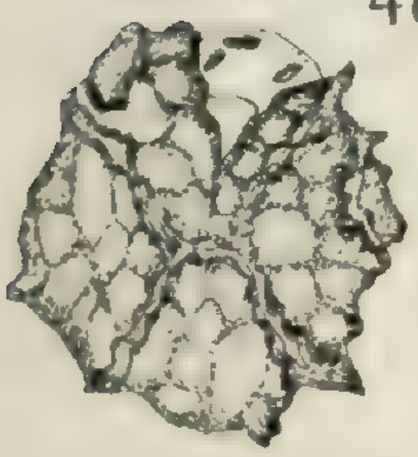
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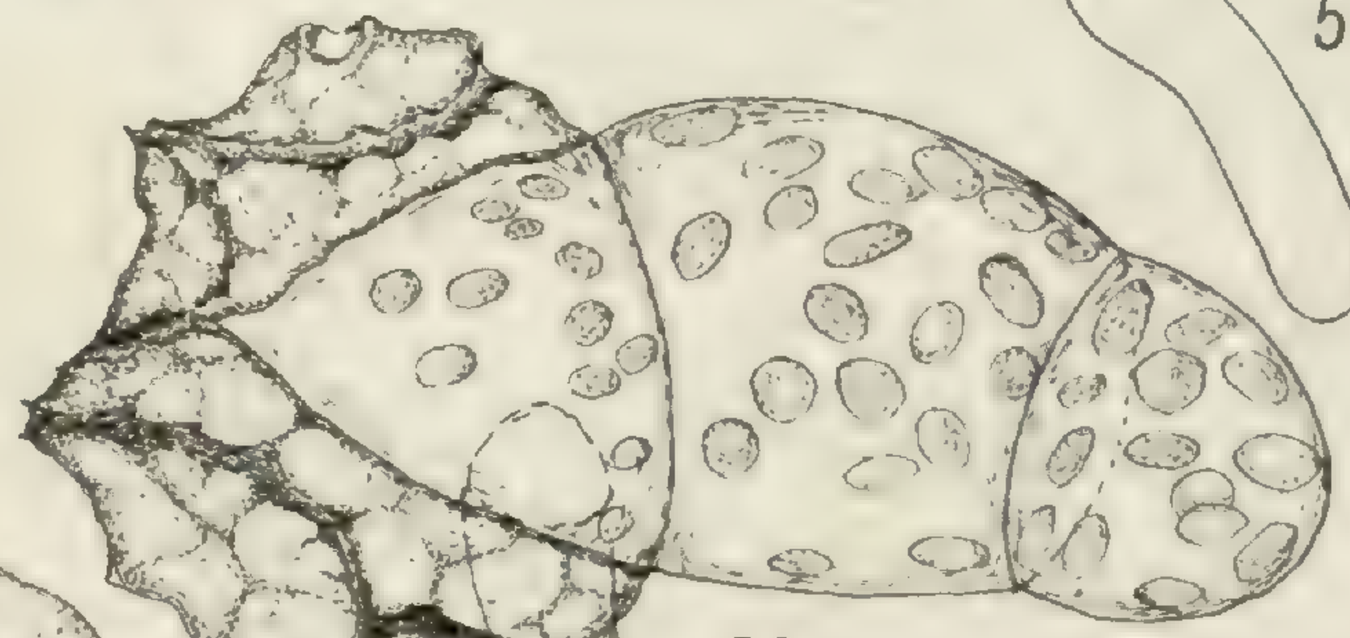
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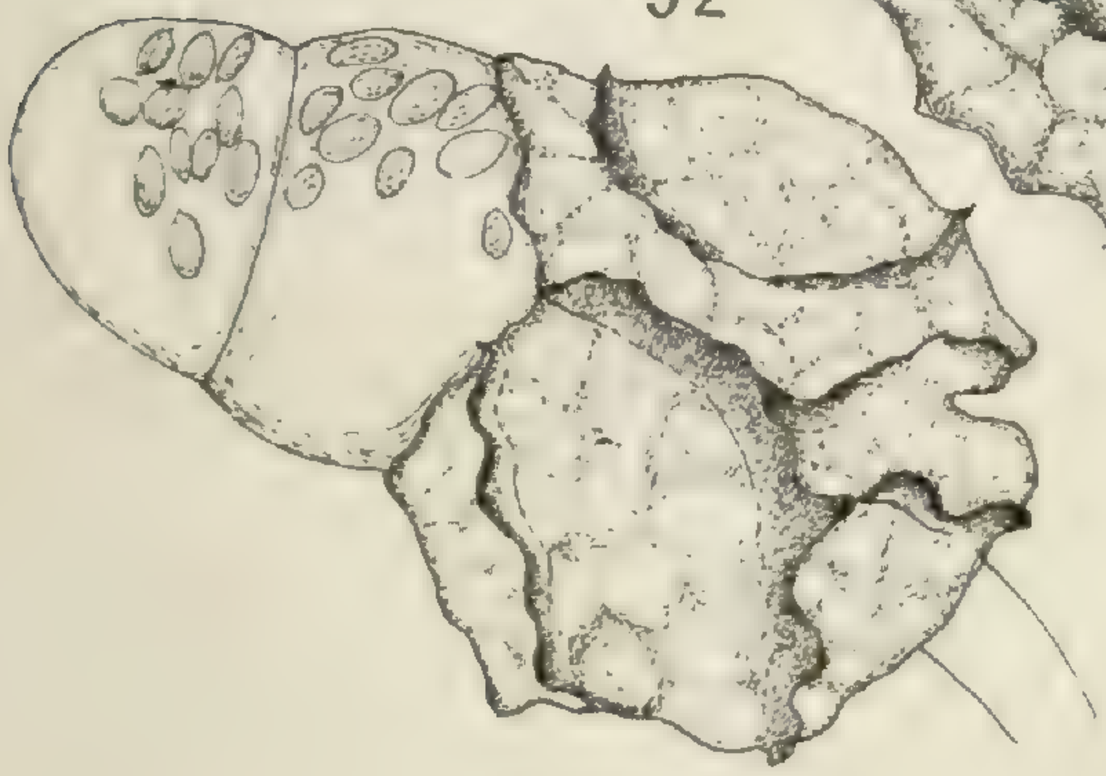
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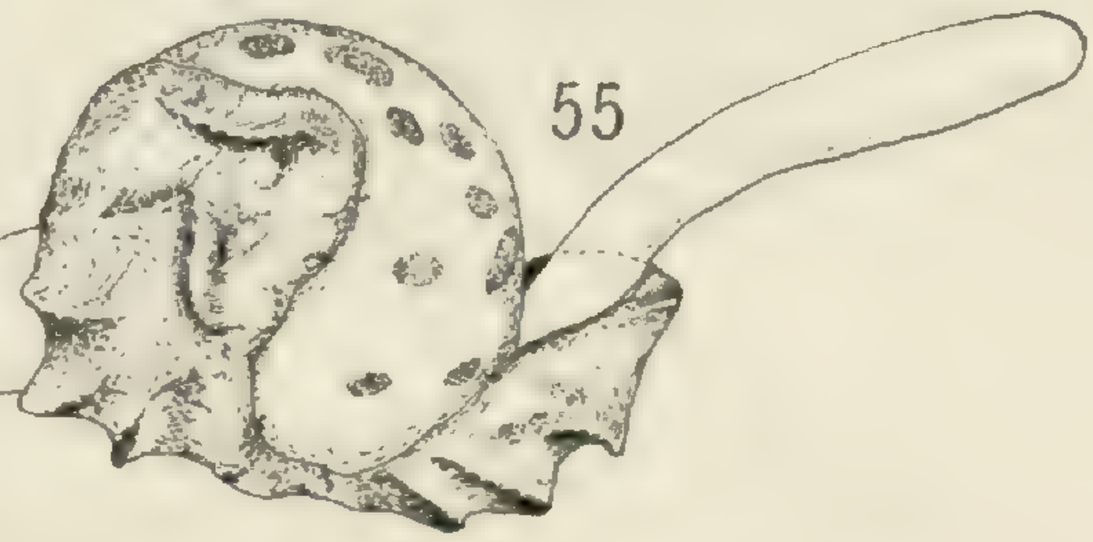
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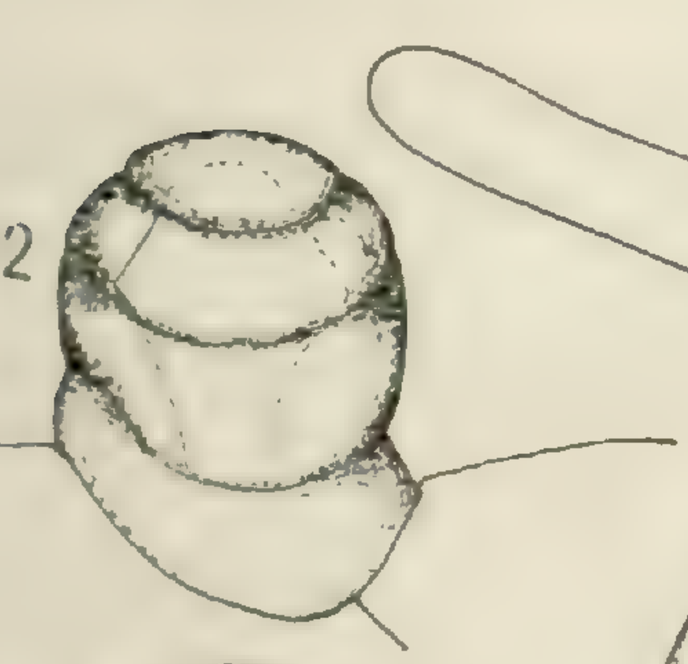
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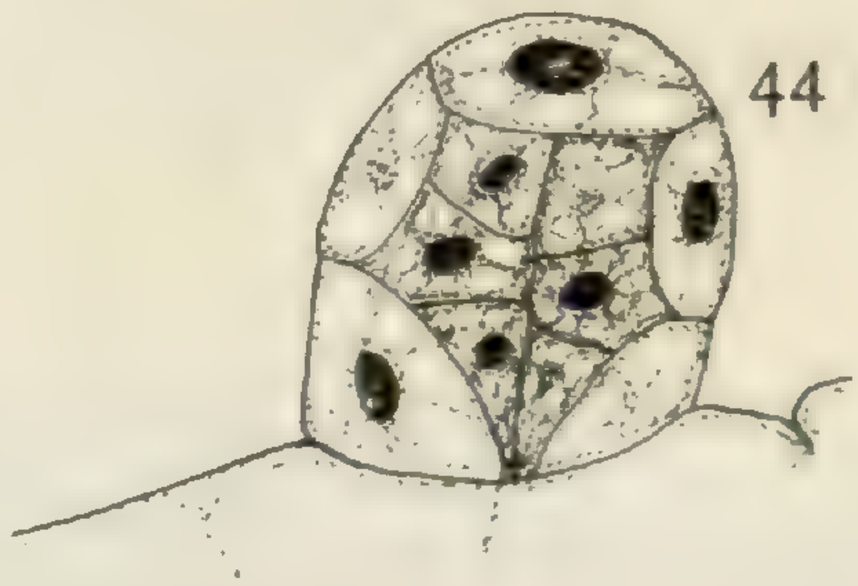
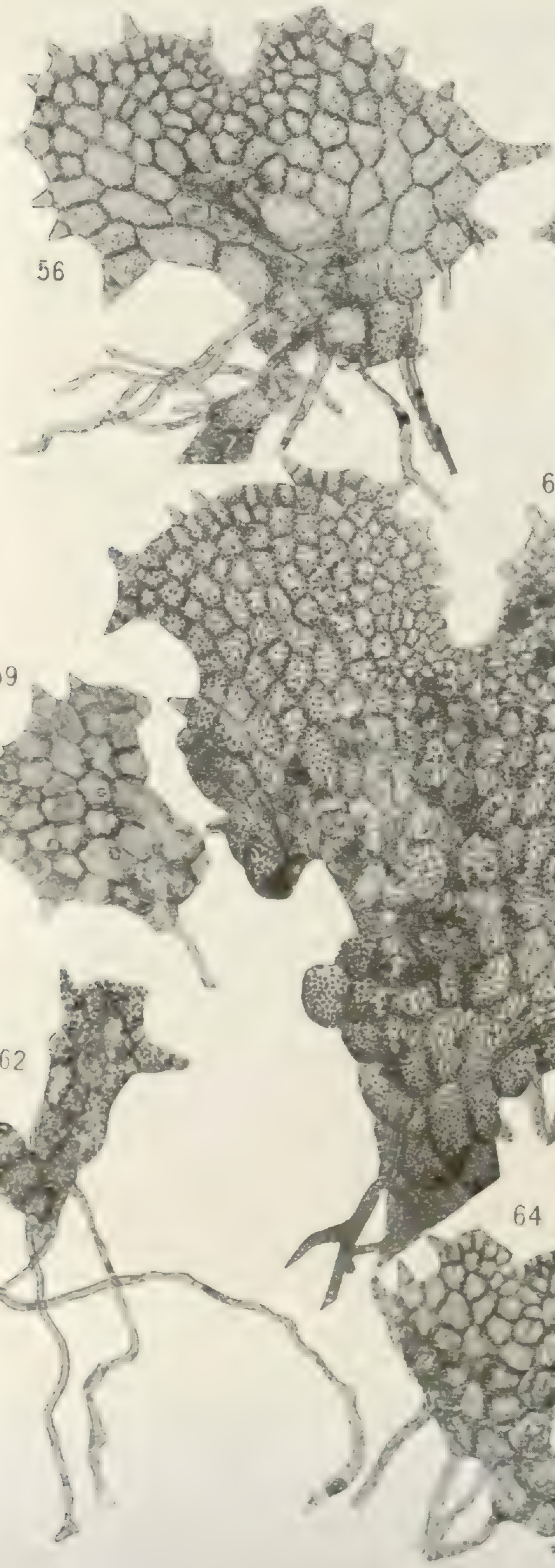
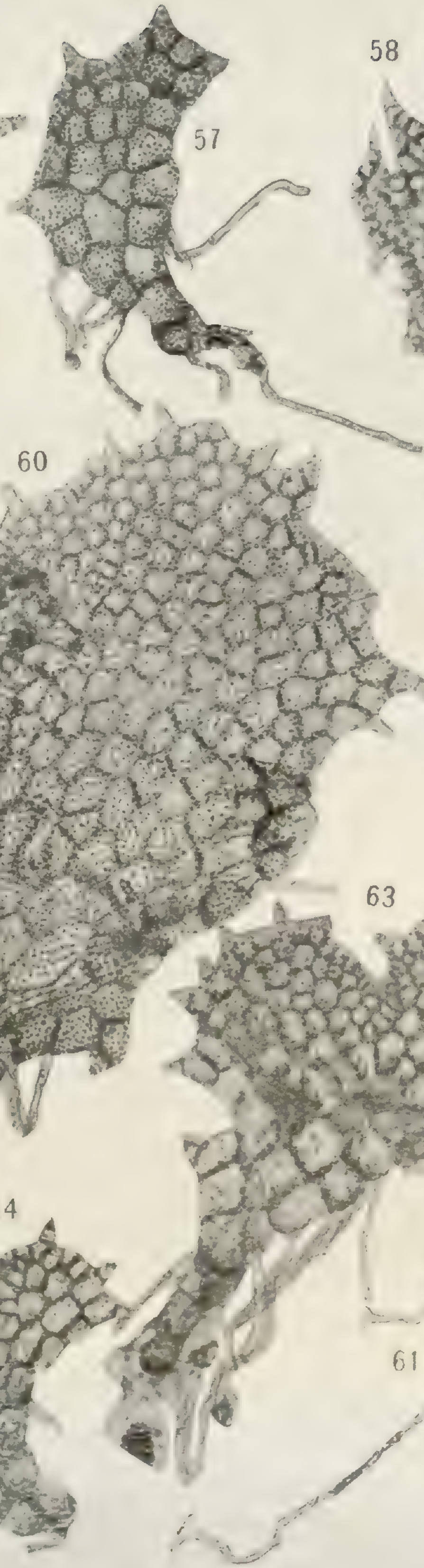
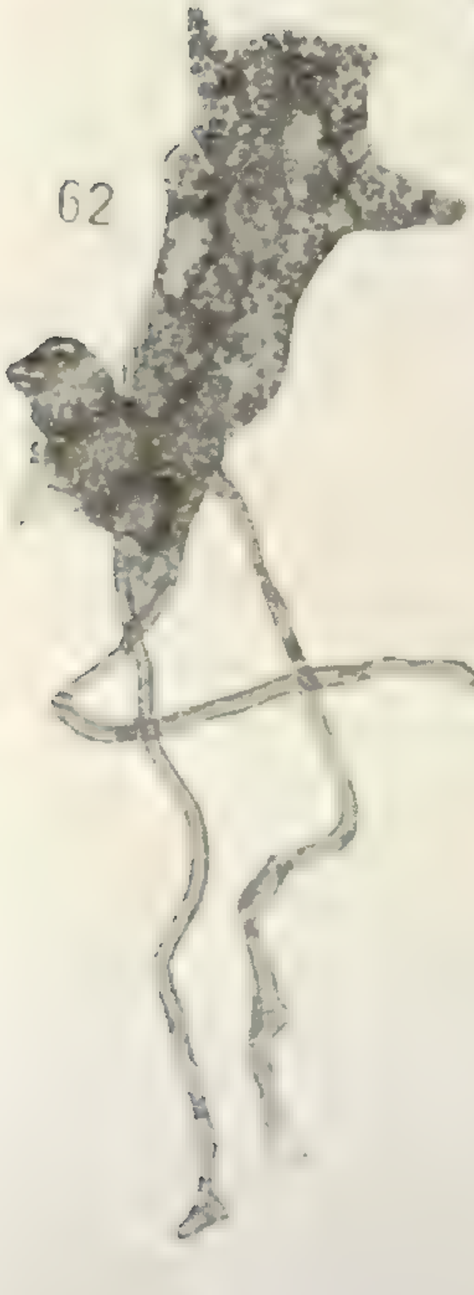
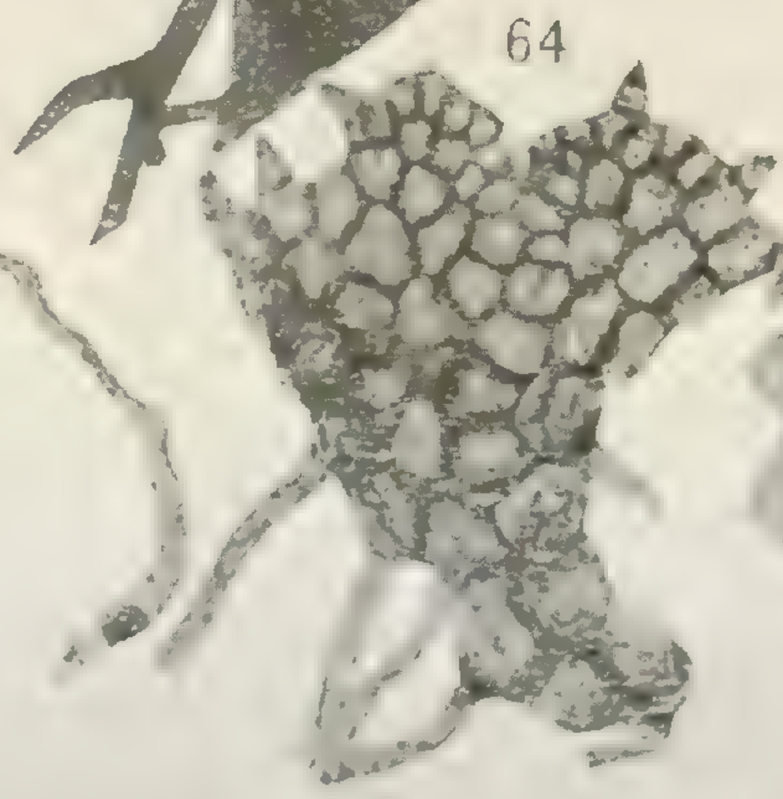

44

PICKETT on CAMPTOSORUS









PICKETT on CAMPTOSORUS

CURRENT LITERATURE

BOOK REVIEWS

Genetics

Some one once said, perhaps more epigrammatically than truthfully, "the progress of a science is in direct proportion to the mathematics used in its development." Whether generally true or not, the constant and rapid progress of genetics since the introduction of Mendel's mathematical notation is a great argument in favor of the statement. At the same time, the chaos that can result from the unwarranted use of mathematics without other premise or analysis is only too familiar to biologists. It has seemed as if those best trained in mathematics were the first to forget that their science is merely a shorthand method of stating the facts, that no more can come out than goes into the mill, though it should come out in a shape more conducive to thorough mental digestion. The slogan of certain biometricians, "there are no premises, all is treatment," has brought many biologists to that state of mind in which they could take seriously POE's sly dig in the "Purloined Letter." In speaking of the necessity of putting oneself in the mental attitude of the thief if the hiding place of the stolen letter were to be discovered, he says: "As poet *and* mathematician, he (the thief) would reason well; as mere mathematician he could not have reasoned at all."

It remained for JOHANNSEN to prove that he is poet, biologist, and mathematician, by showing some four years ago the true relation of KARL PEARSON'S beautiful developments of mathematical methods to genetic research. The motto through the whole 25 chapters of his 500-page book was: "Wir müssen die Erblichkeitslehre mit Mathematik, nicht aber als Mathematik treiben!" JOHANNSEN'S work on the comparative permanence of homozygous types published under the title *Ueber Erblichkeit in Populationen und in reinen Linien* (1903) had already been enthusiastically received by many investigators, partly by reason of the author's mastery of a persuasive style and partly because the conclusions fitted data with which his readers were personally familiar. For these reasons, this elaboration of his ideas met with a cordial reception that is not the fate of many textbooks. But one unfavorable criticism of any importance could be made. The author did not treat adequately the numerous genetic researches in which the problems of heredity had been attacked by methods unlike his own. There is no hesitancy, therefore, in saying that the new edition,[1] with its 30 chapters and 722 pages, to which this criticism may not

[1] JOHANNSEN, W., Elemente der exakten Erblichkeitslehre. Zweite Auflage. 8vo. pp. xi+723. *figs.* 33. Jena: Gustav Fischer. 1913.

be applied with justice (if one excepts cytological research), will be a welcome addition to genetic literature.

In its present form, the work might very easily be divided into two books with separate titles that could be used independently. The one is a thorough introduction to statistical methods as they should be used in the service of biology; the other is a well balanced discussion of the present status of genetic conceptions.

As might be expected, it has been the general discussion of heredity that has received the bulk of the revision; the chapters on biometry were admirably done in the first edition, and the static nature of their substance was such that little change has been necessary. Scarcely a word has been altered in the first five chapters, though CHARLIER'S short method for determining the standard deviation has been added. In chapter 6 the discussion of mean error has been revised and a demonstration from the domain of plant physiology has been added. From this point to chapter 22, only chapters 12 and 13 are new, but the remainder of the book is entirely as written.

In chapter 12 the more recent investigations concerning the possible effect of selection on pure lines are described, while in the next chapter the "misunderstandings" of certain authors who have opposed the theory of permanence of homozygous types are taken up and disposed of with very clear logic, though the style of the rejoinder is sometimes a little caustic.

The last seven chapters of the book are so crowded with information that only a hint as to their contents can be given. They must be read by all who are interested in genetics. Sixty pages are given up to the influence of the factors of environment on variation and 160 pages to Mendelism in its various phases, including heterozygosis, inbreeding, sterility, coupling, and sex determination. Mutations are considered rather concisely in the next to the last chapter, the author being rather of the opinion that the peculiar behavior of *Oenothera Lamarckiana* will ultimately be shown to be the result of segregation and recombination, as has been suggested recently by HERIBERT-NILSSON. The final chapter is a résumé, with observations on eugenics, race hygiene, and evolution.

With reference to the position taken in his earlier work concerning the action of selection, the author remains as firm as a rock. He adds further data of his own to support his position and shows very clearly that the seemingly opposing conclusions of various investigators either are due to fallacious reasoning or are based upon material that is not easily divested of complications that confuse the main issue. To critics who deal only with generalities he makes the following reply that may well be taken to heart by those who deal with evolution from an easy chair:

> Man hat mich kurzsichtig genannt, in Bezug auf die Selektion. Ich konstatiere dies mit Vergnügen; die Prämissen einer oft maszlosen spekulativen Fernsichtigkeit waren ja gerade zu untersuchen und würden wertlos gefunden.

It will doubtless surprise many that JOHANNSEN maintains a firm Lamarckian attitude throughout his book, dealing particularly sympathetically with the work of SEMON. He says: "Man hat mich ferner 'reiner Weismannianer' genannt. Jeder solche 'man' hat mein Buch nicht gelesen oder nicht verstanden." The reviewer must admit, therefore, that he has not understood the author, for after reading the volume he is still firmly convinced that in its essentials it is more nearly Weismannian than Lamarckian. Of course he would not accuse the author of maintaining the morphological hypotheses of WEISMANN with the biophores, determinants, and ids all built into a beautiful structure, but the germ-to-germ inheritance, the dependence of transmissible qualities upon germinal constitution, the invalidity of any particular assumption as to breeding power from the appearance of the soma, and the comparative freedom of the germinal substance from the influence of ordinary environmental changes, as maintained throughout the work, will be classed by most biologists as belonging rightly within the scope of WEISMANN'S conception of heredity.

Very few new terms are introduced by JOHANNSEN in this edition of his book, but two have appeared that seem justified in spite of the abuse that has been showered on the roots used. Individuals that belong to the same phenotype are "isophenous"; individuals that belong to the same genotype are "isogenous." In addition he has adopted WEBBER'S term "clone" for a bud individual.

Taken all in all, one must be very critical to have anything but praise for the new *Erblichkeitslehre*, and it is confidently predicted that it will long remain a classic.—E. M. EAST.

MINOR NOTICES

North American Flora.[2]—Volume 15, part 1, contains the Sphagnaceae by ALBERT LE ROY ANDREWS, the Andreaeaceae by ELIZABETH GERTRUDE BRITTON and JULIA TITUS EMERSON, and the Archidiaceae, Bruchiaceae, Ditrichaceae, Bryoxiphiaceae, and Seligeriaceae by ELIZABETH GERTRUDE BRITTON; part 2 contains the Dicranaceae and Leucobryaceae by ROBERT STATHAM WILLIAMS. New combinations occur in *Sphagnum*, *Ditrichum*, *Dicranella*, *Campylopodium*, *Oncophorus*, *Austinella*, *Leucoloma*, and *Dicranodontium*. New species are described in the following genera: *Dicranella* (2), *Dicranum* (1), *Campylopus* (4), and *Octoblepharum* (1). Volume 22, part 5, is devoted to a continuation of the Rosaceae by PER AXEL RYDBERG and contains the genera *Poterium* to *Rubus* inclusive. New species are described in the following genera: *Agrimonia* (2), *Adenostoma* (1), *Geum* (4), *Sieversia* (1), *Cowania* (1), *Cercocarpus* (7), and *Rubus* (19).—J. M. GREENMAN.

[2] North American Flora. Vol. 15, part 1, pp. 1–75, June 14, 1913; part 2, pp. 77–166, August 8, 1913. Vol. 22, part 5, pp. 389–480, December 23, 1913. The New York Botanical Garden.

Fossil plants.—PELOURDE[3] has contributed the first published volume to the "Bibliothèque de paléontologie," which in turn is one of the 40 divisions of the "Encyclopédie scientifique" under the general direction of TOULOUSE. Under Paleontology, 15 volumes are projected, 3 of which are to be on Paleobotany. The two others will deal with gymnosperms and angiosperms.

The present volume is a compact summary of our knowledge of fossil cryptogams, all but 22 pages being given to pteridophytes. The completeness of the summary may be judged by the fact that the bibliography includes 256 titles.—J. M. C.

Identification of trees.—In order to meet the demands of teachers for a serviceable key for the identification of trees in their winter condition, the authors of *Trees in winter* have reprinted that portion of the volume containing the keys to genera and species.[4] As indicated in the review of the original volume,[5] these keys are based upon the bud, leaf-scar, twig, and occasionally upon the fruit characters. It is anticipated that the convenience of the key in a separate form will be appreciated as an important addition to the equipment for the winter study of our tree flora.—GEO. D. FULLER.

NOTES FOR STUDENTS

Self-sterility.—CORRENS[6] has recently made the phenomena of self-sterility in plants the basis for a searching genetic investigation. After some preliminary experimentation, *Cardamine pratensis* was selected as the material best suited to his purpose, especially as some light had already been thrown upon self-sterility in this species by the investigations of JOST and HILDEBRAND. CORRENS began his study with two specimens of *Cardamine pratensis*, which though derived from the same source (Munster Botanic Gardens) differed markedly in many characters, and were both self-sterile. These two plants (for convenience labeled *B* and *G*) were crossed reciprocally. The offspring, 60 in number, were tested out individually for self-sterility by pollinations (1) from the parents, (2) on the parents, and (3) from sisters. The results are given in such great detail and with such a large amount of easily followed tabular data, that no critic of modern genetic experimental work can criticize the evidence presented on the ground that the details are not all given, or that

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[3] PELOURDE, FERNAND, Paléontologic végétale (Cryptogames cellulaires et vasculaires). 16mo. pp. xxviii+360. *figs. 80*. Paris: Octave Doin et Fils. Fr. 5.

[4] BLAKESLEE, A. F., and JARVIS, C. D., The identification of trees. Key to genera and species from "Trees in winter." 8vo. pp. 16. New York: Macmillan & Co. 1913. 30 cts. For sale only by the authors, Storrs, Conn.

[5] BOT. GAZ. **56**:79. 1913.

[6] CORRENS, C., Selbststerilität und Individualstoffe. Biol. Centralbl. **33**:389–423. *pls. I–II*. 1913.

the language is so technical that transmutations must be made before an ordinary biologist can understand it. When the F_1 plants derived from the crosses ($B\times G$ and $G\times B$) were back-pollinated with B and G, their behavior in reference to seed-setting indicated that they could be separated into four more or less distinct classes: (1) plants fertile with both B and G; (2) plants sterile with both B and G; (3) plants fertile with B, but sterile with G; (4) plants sterile with B, but fertile with G. Numerically, the 60 F_1 individuals were found to distribute themselves among the four classes in about equal proportions, the actual numbers being 16 *bg*: 16 *bG*: 14 *Bg*: 14 *BG*.

Crosses between the individuals of these four F_1 classes, 720 of which were made, in the main confirmed the results obtained through back-crossing with the parents. Crosses between different F_1 plants and plants obtained from foreign sources all resulted in well-filled capsules. From the facts presented, the conclusion that two inhibitors were operating to present self-fertilization was a most natural and simple interpretation. Accordingly, CORRENS gives to those F_1 plants fertile with both B and G, the formula *bg*, indicating the absence of both inhibitors; those plants fertile with one parent and sterile with the other are either *Bg* or *bG*, indicating the absence of one and the presence of the other inhibitor; and lastly, those plants sterile with both B and G are said to be *BG*, indicating the presence of both inhibitors. CORRENS does not dogmatize as to the nature of these inhibitors, except to say that they are hereditary constituents of the germ plasm, which appear to segregate in Mendelian fashion some time prior to the complete development of the egg cells and pollen grains. Hence, self-sterility is not a phenomenon of "Individualstoffe" in the sense in which this term was used by JOST.

In CORRENS' scheme of interpretation, his two original plants are heterozygotes, B represented as *Bb* and G represented as *Gg*, the letters indicating the presence and absence of two distinct factors for self-sterility. *Bb* gives rise to two kinds of gametes, those with the inhibitor (*B*) and those without it (*b*). *Gg* likewise produces gametes with G and gametes from which it is absent (*g*). $Bb\times Gg$ is fertile, but neither $Bb\times Bb$ nor $Gg\times Gg$ would result in seed-formation, as the presence of the factor B would inhibit the growth of the pollen from the *Bb* type. The same is true regarding plants of the *Gg* type. The cross $Bb\times Gg$ results in the four types *BG*, *Bg*, *bG*, *bg*, the classes actually obtained as ascertained by the pollination tests. Types *BG*, *Bg*, and *bG* are self-sterile, while type *bg* should be self-fertile. Plants of the *bg* type should be fertile with the other three types; type *bG* is fertile with only *Bg*; type *bG* with only *Bg*; while *BG* is fertile with only *bg*. It is obvious that types *BB* and *GG* could never be formed.

CORRENS' interpretation of his results opens itself to numerous criticisms, for even the most ardent supporter of Mendelian universality would question the actuality of the four classes. Neither the data from the back-pollination experiments nor the data from those in which F_1 sisters were crossed give any notion of clear-cut classes, such as MENDEL secured in his pea work. For

example, $bg \times bg$ should be fertile, but in the tabular data one finds some *bg* plants giving "alle gut," some giving "alle nichts," and some "3 nichts, 3 gut" in crosses, and, so far as the reviewer can ascertain, no *bg* plant was fertile to its own pollen, each attempt invariably resulting in "alle nichts." The testing out of the 60 F_1 plants ($B \times G$, $G \times B$) with the parents gave similar results. Type $bg \times B$ and $\times G$ gave satisfactory evidence of complete fertility in only about a fourth of the cases, the others varying in proportions of "nichts" and "gut" on each plant tested. CORRENS recognizes these difficulties and only advances his interpretation as a crude, but helpful, working hypothesis. He believes there are many different lines of *C. pratensis*, and that these differ much in genotypical constitution, so that the various irregularities whereby his actual data differ from the theoretical expectation are assignable to this cause, that is to say, there were still other inhibitors at work of which he took no notice.

In support of this conclusion, he points out that "keines der 60 Geschwister war einem anderen oder den Eltern völlig gleich"; also, the results secured by crossing these F_1 sisters with the two foreign races. Another complication encountered by CORRENS was the reaction of the same plant toward the same pollen at different times, at one time pollinations resulting in "gut," at other times "nichts," the result possibly of obscure environmental changes.

CORRENS is to be congratulated on again being a pioneer in opening up a new field to a new viewpoint.

COMPTON[7] in two papers has also contributed to the elucidation of self-sterility phenomena. DARWIN'S observations on the existence of self-fertile and self-sterile races of *Reseda odorata* are confirmed, and in crossing experiments undertaken by this author, the following facts were obtained. Self-sterile × self-sterile gave only self-sterile offspring. Certain self-fertile plants when self-fertilized gave only self-fertile offspring; when crossed with self-sterile plants, the same result was obtained. Other self-fertile plants when self-pollinated gave approximately 3 self-fertile to 1 self-sterile offspring; when crossed with self-sterile plants, the proportion of self-sterile to self-fertile offspring is approximately 1:1. COMPTON tentatively regards self-fertility in *Reseda* as a simple Mendelian dominant, self-sterility being recessive.

In his second paper, COMPTON critically reviews the work of MORGAN, JOST, CORRENS, and other earlier investigators of this phenomenon. Until the investigations of these men, the term self-sterility was a veritable "catch-all." The general notion was extant that a self-sterile plant was fertile with the pollen of every plant of that particular species or race, a condition which all three investigators have shown to be untrue. Many records of self-sterility in species rest on faulty observation; in some cases no evidence was at hand to show that

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[7] COMPTON, R. H., Preliminary note on the inheritance of self-sterility in *Reseda odorata*. Proc. Cambridge Phil. Soc. **17**:7. 1913.

———, Phenomena and problems of self-sterility. New Phytologist **12**:197–205. 1913.

pollen had ever reached the stigma, or that having reached it, favorable conditions for germination were present. *Laburnum vulgare*, as a case in point, remains self-sterile in the absence of slight mutilations produced by insect visitors. Many examples of species with both self-sterile and self-fertile races or varieties are mentioned. An enormous variation in the degree of self-sterility is noted: at one extreme, self-pollination produces but slightly fewer seeds than cross-pollination; while at the other, a few cases are known in which the stigma and pollen of the same flower are mutually poisonous. Environment produces a marked effect on this phenomenon, as often a change in climate changes self-sterile plants to self-fertile ones. *Biophytum sensitivum* is recorded as self-sterile in its open and self-fertile in its cleistogamous flowers. Our knowledge of causes is exceedingly vague and fragmentary. Examination of stigmas fertilized with their own pollen has shown that although germination takes place, the pollen tube is inhibited in its growth in some way so that it never reaches the embryo sac. In JOST's experiments no artificial medium was discovered in which pollen tubes would grow their normal length. COMPTON suggests the presence of a soluble diffusible substance in the stigmatic or stylar tissues which acts in a positive manner toward promoting pollen tube growth. An analogy between self-fertility and immunity, and self-fertility and infection is drawn, in line with the suggestive work of JOST, SCHIFF-GIORGIONI, and others. A special section is devoted to a review of the investigations of BAUR, CORRENS, and COMPTON, on the inheritance of self-sterility, and its racial as opposed to its individual nature. Suggestive analogies are also drawn between self-sterility and certain sexual phenomena, such as non-conjugation and conjugation between different strains in certain species of *Mucor*, *Spirogyra*, and *Dasycladus*. The suggestion is made that so-called sex-differentiation in the Mucorineae may be associated with a simple type of self-sterility; homothallic species would lack inhibitors, while heterothallic species might possess two such inhibitory factors. This paper is replete with suggestion and is a review of incalculable value to all students of general biology.—O. E. WHITE.

The wood of Pinus.—GROOM and RUSHTON,[8] in their detailed account of the wood of the five East Indian pines, have kept several objects in view: the affinities of the species, tropical (hydrophytic) or xerophytic features of the wood structure, relationship of the latter to leaf structure, and the nature of the so-called "bars of Sanio." They have devoted the first part of their work to a general statement and discussion, and in the second part have given a detailed description of each species.

P. excelsa and *P. Gerardiana* belong to the HAPLOXYLON section, having single bundles to the leaves, deciduous sheaths on the spurs, tangential pitting

[8] GROOM, PERCY, and RUSHTON, W., Structure of the wood of East Indian species of *Pinus*. Jour. Linn. Soc. Bot. **41**:457–490. *pls. 24, 25*. 1913. GROOM has also given a brief account of the critical identification of the wood of the five East Indian pines in the Indian Forester **39**:409–411. 1913.

on outermost tracheids of the summer wood, and ray tracheids with almost smooth walls. In *P. excelsa* the needles are in fives and the umbo of the cone scale is terminal. The ray pitting of the tracheids, too, is of the large simple type (Grosseiporen). It thus belongs to subsection CEMBRA, and having long cones and thin cone scales belongs to the *Strobus* group. They note that, "as regards width of spring-tracheids, the American species, belonging to the section HAPLOXYLON that most closely approach it, likewise belong to the group *Strobus*," but draw attention to one difference, the thick horizontal walls of the ray cell of *P. excelsa*, which, according to PENHALLOW, is not characteristic of the CEMBRA type. *P. Gerardiana* has a thick cone scale with central umbo, three leaves to the fascicle, and so belongs to the subsection PARACEMBRA of KOEHNE, which has small ray pitting on its tracheids. Like the other haploxylic forms, it has uniseriate tracheary pitting.

The other three species, *P. longifolia*, *P. Khasya*, and *P. Merkusii*, have 3, 3, and 2 needles, respectively, but "agree not only in the diploxylic nature of the leaves, the persistent nature of the sheath of the dwarf shoot, and the possession of a central umbo on the thick cone-scale, but also in that the transition from spring wood to summer wood is sudden (except in *P. Khasya*), the outermost tracheids of the annual ring do not universally bear pits on the tangential walls, the pits on the radial walls of the spring tracheids are often 2-seriate, and the ray tracheids are denticulate."

The authors consider that the pitting of *P. Merkusii* is of extreme interest, showing a transition between that of the cordaitean or araucarian forms and the ordinary abietineous type. The pits are "in one, two, or three rows, or in peculiar nests, of 3 or 4." These nests are surrounded by "Sanio's rims." The similar condition found by SANIO himself in the root of *P. silvestris* seems to have been overlooked, but at least *P. Merkusii* is the only pine so far described with nests in the stem. They compare this cluster pitting to the well known condition described by PENHALLOW in *Cordaites Newberryii*, and to that in *Cedroxylon transiens* of GOTHAN. These clusters of pits correspond to the "starlike" arrangement which GOTHAN considers as intermediate between araucarian and typical abietinean pitting.

With regard to ecological features, they state: "the tracheids are shortest (3 mm.) in the xerophilous *Pinus Gerardiana*, attain a length of 4 mm. in *P. excelsa* and *P. longifolia*, and 4.6 mm. in *P. Khasya*, and the relatively great length of 7 mm. in the tropical *P. Merkusii*. The size of the tracheids is also commented upon, but a separate article dealing with this point is in process of publication. It has a thick cuticle and hypoderma, much transfusion tissue and "a great development of resin ducts," all in excess of *P. excelsa*. Of the other three they state: "as regards leaf structure, all have stomata on all their faces. *P. longifolia*, the most clearly tending to xerophily, has the thickest cuticle (though not so thick as *P. Gerardiana*), and *P. Khasya* has the thinnest. *P. Merkusii* has the most numerous lines of stomata. . . . . In the more xerophilous *P. longifolia* the endodermis has markedly thickened outer walls,

in *P. Merkusii* this is less marked, while in *P. Khasya* there is no indication of such thickening. All three species contrast with the haploxylic Indian species in the feebler development of the tissue separating the endodermis from the vascular tissue."

These structural ecological results are certainly very interesting, and GROOM's further contribution, which is already in the press, will no doubt add valuable results. It is very desirable that further studies be made on material where the ecological factors are definitely known, and also that a single species be studied under its extreme of wet and dry conditions, in order to determine how much of the change is inherent in the species itself and how much is really due to the external conditions.

The so-called "bars of Sanio" of the Harvard school come in for very severe criticism. They show that these are composed partly at least of pectic compounds, but not of cellulose. They also consider that Miss GERRY mistook SANIO's description of trabeculae for these structures, and propose the term "Sanio's rims" for them, a terminology which is certainly much more in keeping with SANIO's idea ("die Umriss des Primordialtüpfels"). Miss GERRY, however, made a much more serious mistake, for she even quotes SANIO's description of the torus ("diese scheibenförmige Verdickung") as referring to the structures in question.

Resin plates were also found in some of the tracheids adjacent to the rays and also true trabeculae. The authors have also noted the presence of tracheids with bent ends: "when abutting on a medullary ray the end may fork, or bend, so as to run for some distance along the ray and thus form a transition towards a ray tracheid." They also found such tracheids forming radial series apart from the medullary rays.

The detailed description of the species is so arranged that easy reference can be had to any particular feature.—R. B. THOMSON.

Some Jurassic plants.—Among the pteridophytes described by THOMAS[9] from the Marske Quarry of the Middle Jurassic of the Cleveland district of Yorkshire is a new marattiaceous fern, *Marattiopsis anglica*. The genus is "a very common Rhetic and Liassic form, and has been recorded from Sweden, Bornholm, Germany, Poland, and Tongking. Recently two incomplete leaflets from the Jurassic (Kimmeridge) beds of Sutherland have been placed by SEWARD in this genus. Allied forms from the Jurassic of Oregon have been described by LESTER WARD and others under the old name of *Angiopteridium*." Both fertile and infertile pinnae were found. The synangia are considered to have "projected somewhat above the surface, and to have had a fairly firm wall enclosing a number of loculi arranged in two rows; each loculus probably

[9] THOMAS, HUGH HAMSHAW, The fossil flora of the Cleveland District of Yorkshire: I. The flora of the Marske Quarry. Quart. Jour. Geol. Soc. **69**: 223–251. *pls. 23–26*. 1913.

formed a projection, and imparted to the synangium a corrugated appearance." The spores are small, about 0.3 mm., and densely covered with fine projections. "The usual tetrad scar is not seen on any example, but a single straight scar instead, which doubtless indicates that the spores were arranged in the spore mother cells bilaterally and not tetrahedrally."

Of the bennettitalean forms, the staminate sporophylls of *Williamsonia spectabilis* Nathorst are "not uncommon at Marske." THOMAS has been able to make an interesting restoration figure of an almost mature staminate flower of this species. The sporophylls are united into a cup below, and probably in the young condition arched over it, straightening out at maturity. The synangia "lie in regular rows, with their long axis at right angles to the sporophylls," and on the upper or inner side. The synangia of each row appear to be borne on slender stalks. "These stalks seem to have been given off on each side of the central portion of the sporophyll, and may be regarded either as lateral lobes of this organ, or possibly as arising as part of a pinnate structure like the microsporophyll of *Bennettites*, which is adnate with the broad structures hitherto termed sporophylls." He concludes, however, that "whatever may have been the method of production of the synangia of *Williamsonia spectabilis*, this form serves (as NATHORST believes) as a valuable connecting link between the type of microsporophyll seen in *Bennettites* (*Cycadeoidea*), where the sporophyll is a reduced pinnate structure, and the *Williamsonia whitbiensis* type, where the sporophyll is undivided, and bears a double row of synangia on its surface." There is evidence also of the presence of the *whitbiensis* type itself in the Marske beds, and of a female strobilus of *Williamsonia* and other bennettitalean remains. THOMAS' study of the leaves is especially interesting, and also his keenness in distinguishing the bennettitalean from the filicinean forms by microscopic examination of the epidermis, etc. Such critical study, which was inaugurated by NATHORST, puts the results from impressions much more nearly on a par with those from the study of petrifactions.

One of the commonest fossil plants at Marske belongs to the Ginkgoales, *Baiera longifolia*, which has not before been recorded in England. THOMAS has not found a complete leaf, but judges that it must be at least 18 cm. in length. By its great size, and also by its epidermal structure, of which three figures are given, it is distinguished from *B. gracilis*, to which the specimens were previously assigned. He also found *Ginkgo digitata* and *Czekanowskia Murrayana* in the Marske beds.

Of the Coniferales two forms were found, *Taxites zamioides*, of which both upper and lower epidermis are described, and *Elatides* (*Pagiophyllum*) *setosa*. Of the latter THOMAS reported, as his article was in process of publication, that "many specimens of this type have been recently found at Roseberry Topping, bearing male and female cones. These seem to indicate the necessity for creating a new species, and probably a new genus for the form here described."

Further study of the fossil flora of this region promises much for our knowledge of the bennettitalean and coniferous forms.—R. B. THOMSON.

Climatic areas of the United States.—In a recent paper, LIVINGSTON[10] has examined the various climatic data made available through the United States Weather Bureau, and finds that among the factors that may be related to plant growth, only precipitation and temperature have been measured with accuracy, and although the distribution of stations is far from ideal, the resulting data are satisfactory. The evaporation data of the Bureau are shown to be extremely meager, being limited to a single year. From the temperature records LIVINGSTON has made a summation of the daily normal temperatures for the days within the frostless season, for a large series of stations, plotted these upon maps of the United States, and drawn isoclimatic lines. Upon these maps he has also drawn isoclimatic lines of (1) the average daily precipitation, (2) the average daily evaporation, and (3) the differences between the precipitation and evaporation for the same frostless period. The results are three maps in which are delimited areas upon the basis of temperature and upon the basis of a water relation. It is possible in this way to characterize climatically the area occupied by any plant or plant community.

In a previous paper[11] the same author has made a chart of the United States from the records of atmometers at 38 stations widely scattered throughout the country for a period of 15 weeks, extending from May to September, 1908. While there is a general agreement between the isoatmic lines and the limits of the great plant formations of the continent, there are certain marked deviations, one of the chief being in the similarity of the evaporation over much of the prairie region and the eastern deciduous forest. This leads the author to conclude that this prairie region is a potential deciduous forest, a conclusion in accord with the success which has attended tree planting within much of this area and with the advance which the forest is at present making upon the prairie, according to GLEASON and other workers. The mesophytism of the northwestern and northeastern conifer forest centers is demonstrated by average weekly rates of evaporation of only about 100 cc. The deciduous forest occupies a region with a weekly summer rate of from 100 cc. to 200 cc., while a similar rate is shown for the southeastern conifer center, which might indicate that this formation is also potentially a deciduous forest. The semi-arid regions of the southwest show an average of from 300 cc. to 400 cc. weekly.

From these and other data it appears that the summer evaporation intensity furnishes a climatic criterion which is more promising for vegetational studies than any other available meteorological data. It has the further advantage of being rather easily determined by the porous cup atmometer, and such data would also be of great importance in agricultural as well as in ecological investigations.—GEO. D. FULLER.

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[10] LIVINGSTON, B. E., Climatic areas of the United States as related to plant growth. Proc. Amer. Philos. Soc. **52**: 257–275. 1913.

[11] LIVINGSTON, B. E., A study of the relation between summer evaporation intensity and centers of plant distribution in the United States. Plant World **14**: 205–222. 1911.

Cytology of rusts.—In 1912 OLIVE[12] described an intermingling of perennial gametophytic and sporophytic mycelia of *Puccinia obtegens* throughout the host plant. In a new contribution[13] he shows the same condition to hold in two other species, *Puccinia Podophylli* and *Uromyces Glycyrrhizae*. This intermingled growth gives rise to spermagonia, which are followed by aecidiospores and finally by teleutospores in *P. Podophylli*, or by confluent uredosori and teleutosori in *P. obtegens* and *Uromyces Glycyrrhizae*. In the young spring shoots of *P. Podophylli* the order is usually reversed, teleutospores and aecidiospores appearing on the leaf sheaths, and later aecidia and spermagonia on the young leaves.

The mycelium in the leaf sheath is prevailingly binucleate. In the young leaves the uninucleate (gametophytic) mycelium prevails, while in older leaves the binucleate (sporophytic) mycelium becomes predominant. The aecidiospores of *P. Podophylli* and the uredospores of *P. obtegens* and *Uromyces Glycyrrhizae* are all regarded as secondary in origin and thus apogamously derived, arising solely from the binucleate mycelium, as the reviewer[14] formerly pointed out in the first mentioned case. No sexual fusions were found in the young sori in which the mingled gametophytic and sporophytic mycelia occur. The binucleate cells of the sporophyte push in among the uninucleate hyphae of the gametophyte and there form spores directly. OLIVE believes this apogamous condition to be a result of the perennial habit.

The spermatia alone arise from the uninucleate gametophytic mycelium, although binucleate hyphae often invade the immediate neighborhood of the spermagonia. The reviewer, being unaware of this curious mixture of the two kinds of mycelium, in which binucleate hyphae may become predominant, was led to believe the spermatia, like the apogamous aecidiospores, might arise from the binucleate mycelium. The basal cells and spermatia in which he saw more than one nucleus may have been irregularities without particular significance.

Besides the intermingled condition described, two other states of mycelial distribution were observed: (1) an unlimited growth of the perennial sporophytic mycelium alone in *P. obtegens* and *Uromyces Glycyrrhizae*, producing only secondary uredospores and teleutospores in confluent sori, and (2) a localized distribution of the binucleate sporophytic mycelium, giving rise to a sorus of teleutospores in *P. Podophylli*, or in the other two species to the localized "summer generation" or "repeating generation," producing secondary uredospores and teleutospores.—L. W. SHARP.

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[12] OLIVE, E. W., Perennial gametophytic and sporophytic generations in *Puccinia obtegens* (Lk.) Tul. Science **35**:150. 1912.

[13] OLIVE, E. W., Intermingling of perennial sporophytic and gametophytic generations in *Puccinia Podophylli*, *P. obtegens*, and *Uromyces Glycyrrhizae*. Ann. Mycol. **11**:297–311. *pl. 15*. 1913.

[14] SHARP, L. W., Nuclear phenomena in *Puccinia Podophylli*. BOT. GAZ. **51**:463, 464. 1911.

Seedling anatomy of Lupinus.—BECQUEREL[15] has studied the development of the lupine (*L. albus* and *L. luteus*), applying to this plant the "dynamic" method, by which he correlates the diverse results of other botanists and shows how these differences have arisen. He also utilizes his results to combat various current views of the transitional region of the seedling. This "dynamic" method is much in vogue among a group of French botanists, headed by CHAUVEAUD. Instead of taking a seedling of no definite age, plants of all ages and stages in development are studied and compared. This is the method of necessity employed in studying animal development, but has been considered of little importance in plants, because of the slight amount of "making over" of vegetable tissues which is possible. BECQUEREL, however, does claim that a most important change of this nature takes place in the hypocotyl—the *absorption* of the primary wood, which in the young plant comes up very high in the cotyledonary region and in the older plant is not found at all in the same region and only in a vestigial condition at a lower level. This variation in structure has no doubt led, as BECQUEREL claims, to the diverse statements as to the height at which the traces of root structure have been found by different investigators, but that it is due to the absorption of the primary wood and has the phylogenetic significance that BECQUEREL ascribes to it cannot be accepted on the evidence presented. I find no statement to indicate that BECQUEREL has taken into account a very obvious and natural cause for the disintegration of the primary wood. There is a rapid enlargement and elongation of the elements in the hypocotyl, in contrast to the merismatic activity of the more apical parts. In this growth the tracheary elements naturally cannot take part, and in consequence become dissociated and disorganized, their function being assumed by the secondary elements which now appear. Again, BECQUEREL'S statement that these elements have disappeared by reason of absorption, as in animal tissues, postulates the presence of an enzyme such as hadromase, and this has not been shown to occur in any plants except the xylophilous fungi.

The objections that BECQUEREL raises to the current views of the transition region between stem and root will lead, no doubt, to clearer ideas and wording of the subject in future texts.—R. B. THOMSON.

Sea-water and the distribution of plants.—The discovery of any efficient means of determining in a quantitative manner the factors limiting the extent and composition of various plant associations must be regarded as an important contribution to ecology. In salt marshes the concentration of salt in the soil water has long been regarded as a limiting factor, and now HARSHBERGER[16] has

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[15] BECQUEREL, P., L'ontogénie vasculaire de la plantule du lupin. Ses conséquences pour certaines théories de l'anatomie classique. Bull. Soc. Bot. France **60**: 177–186. *pl. 4. figs. 5.* 1913.

[16] HARSHBERGER, J. W., An hydrometric investigation of the influence of sea-water on the distribution of salt marsh and estuarine plants. Proc. Amer. Phil. Soc. **50**:457–496. *pls. 2. figs. 7.* 1911.

measured this concentration by means of a convenient type of hydrometer, made the necessary corrections for temperature, and has been able to determine the limiting degrees of salinity which the dominant salt marsh plants of the New Jersey coast are able to withstand. Among the species able to grow in water containing over 1 per cent of sodium chloride, *Salicornia herbacea*, *Distichlis spicata*, and *Limonium carolinanum* seem to have the smallest range of accommodation; while *Spartina stricta maritima*, *S. patens*, and *Juncus Gerardi* show the widest limits. *Typha angustifolia* cannot grow in water that approximates a sodium chloride content of 1 per cent, and in much less dilute solutions shows the detrimental effect of the salt. From a large number of determinations, the height of the plants and the size of their spikes are shown to vary inversely with the concentration of the water in which they developed, the optimum condition being entirely fresh water. The paper contains other valuable data and points the way for intensive studies of the vegetation of salt marshes and alkaline soils.

A few notes on the deposits shown in sections of the salt marsh soil indicate a definite succession in the former vegetation similar to that at present in progress, and that there has been a progressive submergence of the marsh either from a change of tidal level, as held by JOHNSON,[17] or from a general subsidence of the entire coast line, as is believed by most investigators.—GEO. D. FULLER.

Queensland ferns.—Dr. F. M. BAILEY, the veteran colonial botanist of Queensland, Australia, has just published an interesting review[18] of DOMIN'S work on Queensland plants, so far as it concerns ferns and fern allies.[19] The new species and varieties, as well as species new to Queensland, are listed, often with descriptions. The notable feature of the review is the almost constant criticism of DOMIN'S new species and varieties. For example, in regard to *Psilotum triquetrum* Sw. var. *fallacinum* Domin, he says that "the distinctions given seem only those of growth and situation"; *Selaginella flabellata* F. v. M. var. *brevispica* Domin "is scarcely worthy of a distinctive name"; *Marattia oreades* Domin "can hardly be separated from that very variable species *M. fraxinea* Sm."; in regard to two new varieties of *Platycerium alcicorne* and one new variety of *P. grande* he says that "it is scarcely advisable to attach names to isolated plants of *Platycerium*, particularly as differences in their growth and form are so often caused by situation." "Finally," says BAILEY, "I think that

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[17] JOHNSON, D. W., The supposed recent subsidence of the Massachusetts and New Jersey coasts. Science N.S. **32**:721–723. 1910; also Botanical evidence of coastal subsidence. Science N.S. **33**:300–302. 1911; also BOT. GAZ. **56**:449–468. 1913.

[18] BAILEY, F. M., Contributions to Queensland flora. Bot. Bull. no. 17. pp. 14. 1913.

[19] DOMIN, K., Beiträge zur Flora und Pflanzengeographie Australiens I. Abt. Pteridophyta (Prodromus einer Farnflora Queenslands). Stuttgart. 1913.

had Dr. DOMIN had a longer experience with our ferns, and observed the great diversity of their form and growth, he would have realized that some of his new kinds were but growths of well known species."

Nearly 70 years of experience with plants in the field, much of it with Queensland plants, add to the weight of BAILEY'S comments. At the time of DOMIN'S visit, the present reviewer was collecting morphological material in Queensland, and can heartily agree with the remarks regarding the variability of *Psilotum*, *Marattia*, and *Platycerium*. In *Platycerium*, particularly, the appearance of the plant is so affected by its position on the tree, that isolated plants might be described as new species, if the differences were not so obviously due, in some cases, to merely mechanical causes.—C. J. CHAMBERLAIN.

A new seed genus.—Miss BENSON[20] has made *Conostoma ovale* Williamson the basis of a new form genus, which she calls *Sphaerostoma*. Along with *C. ovale* the doubtful species *C. intermedium* is included as *S. ovale*. In general structure the seed resembles *Lagenostoma*, and is found most frequently without its cupule (or outer integument), from which it doubtless separated when dropped. All of the epidermal cells of the integument are papillate, and at the apex, where the integument is free from the nucellus, they become so elongated as to form a whorl of epidermal crests, the "canopy" being 8-lobed. These crests Miss BENSON calls "frills."

The structure of the lagenostome, however, is quite peculiar. There is the usual central column of nucellar tissue, surrounded by the moatlike pollen chamber which is invested by the epidermis of the nucellus. But the roof of the pollen chamber is modified into what Miss BENSON regards as "an elastically acting mechanism" which definitely closes the pollen chamber after there has been a dehiscence which admits the pollen grains. This elastic mechanism, forming the roof of the pollen chamber, is the epidermis so modified as to resemble in appearance a multiseriate annulus. The conclusion is that in dehiscence this mechanism straightens elastically, admits pollen grains, and then closes the chamber again.

The seeds are provisionally referred to *Heterangium Grievii* on what seems to be excellent evidence, namely constant association, suggestions of actual continuity of ovules with the parent plant, and the evident morphological relationship to *Lagenostoma*.—J. M. C.

Culture of Opuntia.—GRIFFITHS[21] records some very interesting observations upon the behavior of certain species of *Opuntia* under culture. The plants were grown at the government stations at Brownsville and San Antonio,

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[20] BENSON, MARGARET J., *Sphaerostoma ovale* (*Conostoma ovale* et *intermedium* Williamson), a Lower Carboniferous ovule from Pettycur, Fifeshire, Scotland. Trans. Roy. Soc. Edinburgh **50**: 1–15. *figs. 3. pls. 1, 2.* 1914.

[21] GRIFFITHS, D., Behavior, under cultural conditions, of species of Cacti known as *Opuntia*. U.S. Dept. Agr., Bur. Pl. Ind., Bull. no. 31. pp. 24. *pls. 1–8. fig. 1.* 1913.

Texas, and Chico, California. At each of these places between 600 and 1500 varieties of *Opuntia* were under cultivation. The general purpose of the investigations was economic, but incidentally the behavior of these plants was very suggestive. Spiny plants grown near the coast of Texas mature spines much more slowly than in drier regions. Changes in the environment seem to have more effect on the production of spicules (bristles) than of spines, the evidence indicating that conditions unfavorable to vegetative production stimulate the growth of spicules. An interesting contrast between the conditions in Southern Texas and California is shown by the fact that the former region is favorable for the production of vegetative growth, while California is better adapted to fruit production. It was found that many species are not promising for breeding purposes because of lack of variability; they are very constantly spiny, and spine protection seems to be directly proportional to plant vigor. The power of recovery from the effect of low temperature is remarkable, especially when the succulency of the plant is considered. A very interesting observation was that in many of the species, especially the larger ones, the plants grown from cuttings and those grown from seeds are very different in appearance; the latter are tree-like and the former are headed on the ground without distinct stems.—J. M. C.

Mitosis in Conjugatae.—In agreement with the earlier work of LUTMAN, VAN WISSELINGH[22] finds that the nuclei of *Closterium* show at all stages an essential correspondence with those of higher plants. Division is strictly mitotic, and the chromosomes, more than 60 in number and of various lengths, all come directly from the reticulum, which is composed of but one material. They are not placed in a ring around a central spindle at metaphase, as LAUTERBORN thought, but form a uniform plate of the usual type. VAN WISSELINGH denies the presence of a continuous spirem at prophase and telophase as reported by LUTMAN. The nucleolus is not of the peculiar kind previously described for *Spirogyra;* in *C. Ehrenbergii* it is really an agglomeration of many small nucleoli. No centrosomes were found.

In *Eunotia*,[23] also, the nucleus divides mitotically, as in other diatoms, but well developed chromosomes are not formed. The nuclear reticulum gives rise to small irregular bodies which become arranged in the form of a "nuclear plate" around the characteristic "central spindle" of the diatoms. The nuclear plate divides to form two daughter plates. VAN WISSELINGH's results here agree with those of KLEBAHN and KARSTEN on other diatoms, but not with those of LAUTERBORN, who found in several species long and well developed chromosomes in both mother and daughter nuclei.

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[22] VAN WISSELINGH, C., Über die Kernstruktur und Kernteilung bei *Closterium*. Siebenter Beitrag zur Kenntnis der Karyokinese. Beih. Bot. Centralbl. **29**[1]:409–432. *pl. 10.* 1913.

[23] VAN WISSELINGH, C., Die Kernteilung bei *Eunotia major* Rabenh. Achter Beitrag zur Kenntnis der Karyokinese. Flora **105**:265–274. *pl. 10.* 1913.

The value of these contributions is unfortunately impaired by the small size and diagrammatic character of the illustrations.—L. W. SHARP.

Morphology of Tetraclinis.—SAXTON[24] has investigated *Tetraclinis articulata*, the "gum sandarach" tree of Morocco and Algeria. He has given an unusually detailed account of microsporogenesis. The mature pollen grain is uninucleate, and approximately three months elapse between pollination and fertilization; while it is 12 months from the first appearance of the strobili to the complete maturity of the seeds. The development of both gametophytes resembles that of other *Cupressus* forms. Wall-formation in the proembryo occurs in passing from the four-nucleate to the eight-nucleate stage, the mature embryo being somewhat variable in the number and arrangement of the cells. More than one tier of cells takes part in suspensor formation. In the mature embryos three, four, and five cotyledons were found. One of the interesting cytological features is the segregation of the chromosomes into two groups in the prophase of the first division of the fertilized egg. This is taken to be an evidence of the continued individuality of male and female chromosomes. The chromosome numbers were found to be 12 and 24. SAXTON is inclined to believe that the *Callitris* group was derived from the *Cupressus* group through some plant resembling *Tetraclinis*. He traces a geographic line of evolution from northern to southern Africa, and thence by means of former antarctic land connection to Australia. This would make *Widdringtonia* the most primitive of the *Callitris* forms.—J. M. C.

The absorption of water by aerial organs.—It is now pretty generally believed that the absorption of water by the aerial organs of vascular plants is rarely a thing of consequence outside of the Bromeliaceae, although it has been known for a long time that flaccid leaves immersed in water recover their turgescence. Experiments made by various investigators have shown that the cell sap of *Salicornia* and other salt marsh plants has an osmotic pressure considerably above that of sea water. Hence the question arose with Miss HALKET as to the possibility of such plants absorbing water when immersed at high tide. It was found that the aerial organs of *Salicornia* plants can absorb water from a 3 per cent solution of sodium chloride, and a larger amount from distilled water.[25] As might be expected, the amount absorbed is greatly increased if the plants are allowed to transpire freely before immersion, without being able to absorb water through the roots. Experiments made on non-halophytic plants under similar conditions resulted in a loss of water rather than in absorption, hence it is concluded that the absorption noted in the salt marsh plants

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[24] SAXTON, W. T., Contributions to the life-history of *Tetraclinis articulata* Masters, with some notes on the phylogeny of the Cupressoïdeae and Calltroïdeae. Ann. Botany **27**:577–605. *figs. 9. pls. 44–46.* 1913.

[25] HALKET, ANN C., Some experiments on absorption by the aerial parts of certain salt-marsh plants. New Phytol. **10**:121–139. 1911.

is due to the high osmotic pressure. It is suggested that in these plants absorption by aerial organs (involving both sea water and atmospheric water) may in nature supplement root absorption, although the latter doubtless is the more important.—H. C. COWLES.

Xerophytic fern prothallia.—The very delicate character of fern prothallia in general and their usual development under humid conditions have made it difficult to account for the establishment of various ferns in dry, rocky situations. Some light has recently been shed upon the subject by experimental cultures of the prothallia of *Camptosorus rhizophyllus* by PICKETT.[26] Exposed to conditions of desiccation arranged to simulate as far as possible those of dry limestone ledges under conditions of natural drought, the prothallia showed complete recovery after 34 days, and 25 per cent recovery after 55 days. Under rather more rigorous conditions, only 50 per cent of the prothallia died after 38 days' exposure to conditions of drought that killed all the prothallia of *Onoclea Struthiopteris* in 48 hours; while under the most rigorous aridity in a sulphuric acid desiccator a small proportion of the prothallia survived 4 days' exposure. All these tests go to prove that the drought-resisting character of the prothallia of *Camptosorus* must be a very important factor in the establishment of this fern in its characteristically xerophytic habitats.—GEO. D. FULLER.

Rhodophyceae of the Indian Ocean.—Mrs. WEBER VAN BOSSE[27] has reported upon a collection of Rhodophyceae made in 1905 on the Percy Sladen Trust Expedition to the Indian Ocean. The geographical distribution shows a great resemblance between the algal flora of the Indian Ocean and that of the Malay Archipelago, as well as that of the east coast of Africa. A table shows the locality, bottom, depth, and distribution of each of the 79 species collected, among which there are 18 new species, and a new genus (*Pseudenosiphonia*) of Rhodemelaceae.—J. M. C.

Plant phyla.—BESSEY[28] has revised his *Synopsis of plant phyla*, making many changes in the arrangement of the orders and families. He recognizes 14 phyla, and gives the latest approximate enumeration of the known species of plants as 233,614. These species are distributed among the current large groups as follows: Thallophytes 79,450, of which the Ascomycetes and Basidiomycetes include 64,000; Bryophytes 16,600; Pteridophytes 4,524; Gymnosperms 540; Angiosperms 132,500.—J. M. C.

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[26] PICKETT, F. L., Resistance of the prothallia of *Camptosorus rhizophyllus* to desiccation. Bull. Torr. Bot. Club. **40**:641–645. 1913.

[27] WEBER VAN BOSSE, Mrs. A., Marine algae, Rhodophyceae, of the "Sealark" expedition, collected by Mr. J. STANLEY GARDINER. Trans. Linn. Soc. London Bot. II. **8**:105–142. *pls. 12–14.* 1913.

[28] BESSEY, CHARLES E., Revisions of some plant phyla. Univ. Studies **14**[1]: 73. 1914. Lincoln, Nebraska.

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THE
BOTANICAL GAZETTE
APRIL 1914

THE EFFECT OF SHADING ON THE TRANSPIRATION AND ASSIMILATION OF THE TOBACCO PLANT IN CUBA

HEINRICH HASSELBRING

(WITH ONE FIGURE)

Introduction

This paper gives an account of experiments conducted in Western Cuba for the purpose of determining the effect on transpiration and assimilation in the tobacco plant of the cheese-cloth shade which is frequently used in that region for shading tobacco. A comparative study of the transpiration and assimilation of the tobacco plant under normal conditions and under the conditions induced by cheese-cloth shading is of interest for several reasons. First, although investigations[1] of the influence of different light intensities on transpiration have nearly always led to the conclusion that the rate of transpiration is decreased with a lessening illumination, the experiments which have established this conclusion have necessarily been conducted with plants or parts of plants which could be kept under observation only for short periods of time, and often under laboratory conditions. Data permitting a comparison of the transpiration of plants under normal conditions with others of the same kind shaded during their entire development are not at

[1] KOHL, G., Die Transpiration der Pflanzen, etc. pp. 52–74. 1886; BURGERSTEIN, A., Die Transpiration der Pflanzen. pp. 85–103. 1904; LIVINGSTON, B. E., Light intensity and transpiration. BOT. GAZ. **52**:417–438. 1911.

hand.[2] Second, in view of the fact that a large portion of the tobacco crop is grown regularly under shade, such data would not be devoid of practical interest, especially in regions like Western Cuba, where annual crops require irrigation and where much of the irrigation is accomplished by toilsome hand labor. Finally, in the minds of investigators, transpiration has frequently been associated with assimilation. The water requirement of agricultural crops has usually been stated in a ratio of the quantity of water transpired to dry substance produced. As a rule, this ratio has been considered merely as a convenient empirical expression of the water-utilization of plants; but some writers have assumed a closer relation and have postulated a direct influence of transpiration on production, or conversely. Therefore, it is of interest to determine to what extent the conditions induced by shade, either directly or through their influence on transpiration, affect production.

For these reasons, the work described in the following pages was undertaken at the Cuban Agricultural Experiment Station at Santiago de las Vegas during the season of 1908–1909. For constant and ready aid in carrying out the exacting work required for this investigation, I am much indebted to ENRIQUE IBAÑEZ and AUGUSTIN GARCIA, at that time my assistants at the station.

Environment

GENERAL STATEMENT

For the purpose of these experiments, six tobacco plants were grown in the open ground, and six under cheese-cloth shade in a manner described later. The cheese-cloth was of the kind generally used in Cuba and elsewhere for shading tobacco (fig. 1). During the middle of the day, when the sun's rays are nearly perpendicular, this cloth casts a barely perceptible shadow, which, however, is more noticeable early in the morning or late in the afternoon. In

[2] FITTBOGEN indeed grew to maturity some plants of oats in a greenhouse and others in the open. He regarded the loss of light due to its passage through the glass as one of the factors tending to lower the transpiration of the plants. This experiment, however, can hardly be classed as a study of the effect of shading on transpiration. FITTBOGEN, J., Über Wasserverdunstung der Haferpflanze unter verschiedenen Wärme-, Licht-, und Luftfeuchtigkeits-Verhältnissen. Landw. Jahrb. **3**:141–146. 1874.

order to determine the effect of the cheese-cloth on the environment of the plants, measurements were made during the course of the experiments of the various environmental factors both under the

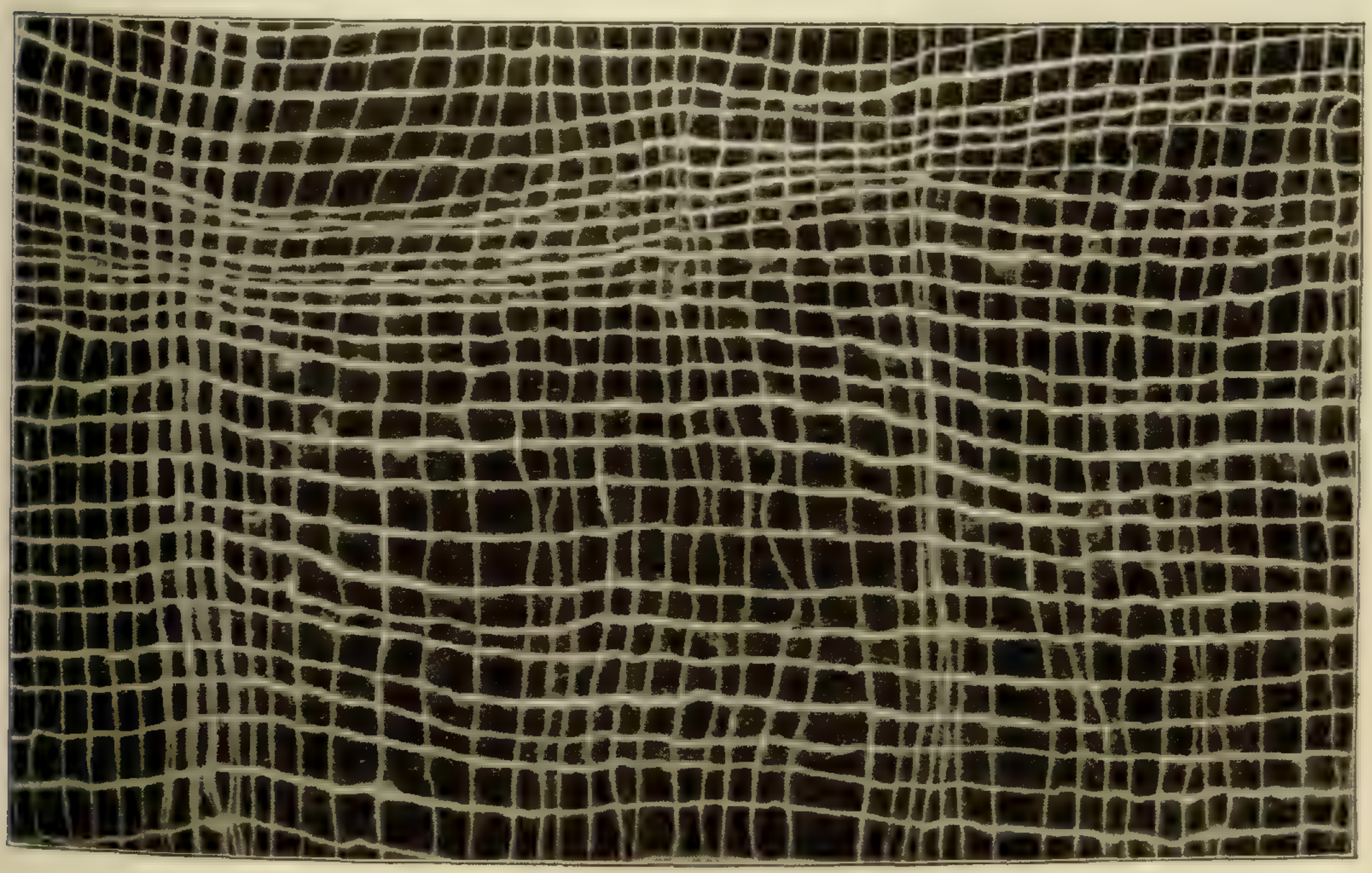

FIG. 1.—Photograph of the cheese-cloth used in the experiments; natural size

cheese-cloth and in the open. The results of these measurements are given in the following paragraphs.

LIGHT

The light intensity under the two conditions was measured by the photometric method. The method in general was that described by WIESNER[3] and used by him in his extensive researches on the light relations of plants. However, owing to the difficulty of making a sensitive paper which at some stage in the process of darkening would exactly match the normal tint kindly sent the writer by Professor WIESNER, a standard instrument, the Wynne exposure-meter, was used in these observations. The time of exposure was measured by means of a stop-watch. The applicability of this method, as well as its shortcomings, have been fully discussed by WIESNER and others, and recently LIVINGSTON[4] has subjected

[3] WIESNER, J., Der Lichtgenuss der Pflanzen. pp. 10–33. 1907.

[4] LIVINGSTON, *loc. cit.*

to a critical comparison this and other methods of measurement of solar energy. These discussions need not be repeated here. In spite of the shortcomings of this method, it has the advantage of being capable of easy manipulation and gives sufficiently correct results for a comparison of relative light values under the cheese-cloth shade and in the open.

The observations were taken on December 12, December 23, and January 25. December 12 and January 25 were bright days with only a few hazy clouds, which are usual in Cuba toward the middle of the day. December 23 was cloudy, so that all the light on that day was diffuse. On the first day, December 12, ten observations were taken usually at each hour period for each kind of light under each condition, but on account of the length of time required to make that number of observations and the change of light meanwhile, only five observations were taken at each reading on the other days. As a rule, the observations were taken alternately within and without the tent. Since the personal equation in the judgment of color is likely to play an important part in determining the time required for the sensitive paper to reach a standard tint, the probable error of the average was calculated for each set of observations, except some taken early or late in the day, when, on account of the weakness of the light, it was not possible in some cases to take more than one or two observations. An examination of the probable errors given with the column of averages shows that it is not of undue magnitude. It may also be stated that the ten or five observations from which the average is made up showed a very close agreement, usually within a fraction of a second of each other except when the exposures were very long, that is, 30 seconds or more, although, as has been stated, the observations were taken alternately in the two stations in such a way that the observer was not influenced by the previous observation and record. From the observations thus made each hour in each station, the average time of exposure was calculated. These averages with their probable errors are given in table I.

The light intensity is of course proportional to the reciprocal of the time of exposure. These reciprocals were obtained, therefore, but in order to reduce the figures of each day to relative values, the

highest light intensity for each day was assigned the value of 10, and the other values were reduced to the same basis. These figures are given in table II. The relative light values for any one day, therefore, are all directly comparable, but the figures for one

TABLE I

AVERAGE LENGTHS (IN SECONDS) OF EXPOSURES MADE IN DETERMINING LIGHT VALUES

TIME OF EXPOSURE	OPEN		SHADE	
	Total light	Diffuse light	Total light	Diffuse light
December 12				
8:15– 8:30 A.M. .	5.33±0.12	10.66±0.10	8.72±0.10	12.13±0.25
9:15– 9:30 A.M. .	3.56±0.07	6.90±0.13	6.22±0.11	9.72±0.13
10:00–10:15 A.M. .	2.92±0.09	7.58±0.18	4.36±0.10	7.31±0.11
11:00–11:15 A.M. .	2.23±0.05	8.26±0.13	3.29±0.02	9.32±0.12
12:30– 1:00 P.M. .	2.32±0.06	7.55±0.13	3.06±0.11	9.29±0.10
1:30– 2:00 P.M. .	3.58±0.07	9.67±0.10	5.13±0.07	10.48±0.21
2:30– 3:00 P.M. .	5.16±0.11	12.30±0.23	7.14±0.19	10.33±0.12
3:30– 4:00 P.M. .	8.70±0.18	17.26±0.18	11.34±0.12	15.48±0.34
4:45– 5:15 P.M. .	24.32±0.95	60.00	134.30	.....
December 23				
7:00 A.M.	.....	33.07±0.86	.....	56.33±1.03
8:00 A.M.	.....	10.68±0.89	.....	15.68±0.57
9:00 A.M.	.....	6.46±0.12	.....	9.23±0.49
10:00 A.M.	.....	7.72±0.18	.....	12.52±0.12
11:00 A.M.	.....	6.76±0.41	.....	8.80±0.50
12:00 M.	.....	10.68±0.17	.....	13.66±0.18
1:00 P.M.	.....	5.14±0.08	.....	7.84±0.08
2:00 P.M.	.....	6.34±0.16	.....	8.44±0.08
3:00 P.M.	.....	6.96±0.08	.....	10.56±0.11
4:00 P.M.	.....	14.36±0.28	.....	21.40±0.34
5:00 P.M.	.....	87.50±3.04	.....	94.00±0.67
January 25				
7:00 A.M.	59.50±2.36	.....	71.00±4.05	.....
8:00 A.M.	8.80±0.06	.....	13.65±0.10	.....
9:00 A.M.	3.60±0.07	8.48±0.08	7.12±0.12	11.20±0.10
10:00 A.M.	3.00±0.06	10.32±0.14	6.32±0.10	11.60±0.20
11:00 A.M.	2.34±0.09	6.88±0.16	4.76±0.11	7.82±0.07
12:00 M.	2.04±0.05	6.92±0.16	3.60±0.08	8.16±0.08
1:00 P.M.	2.48±0.05	7.68±0.09	4.00±0.06	8.56±0.16
2:00 P.M.	3.52±0.08	10.08±0.12	5.44±0.10	11.35±0.17
3:00 P.M.	5.60±0.21	13.12±0.29	8.12±0.09	14.68±0.22
4:00 P.M.	7.20±0.09	20.00±0.30	18.48±0.26	.....

day are not directly comparable with those of another day, since for each day a different number was taken as the unit. This system was adopted since it was not the purpose to compare the light values of the different days, but only those within the cheese-cloth tent with those outside.

From table II the following conclusions may be drawn: On the bright days (December 12 and January 25) the total light within the tent was 30–40 per cent, or about one-third, less than that with-

TABLE II

RELATIVE LIGHT VALUES UNDER CHEESE-CLOTH SHADE AND IN THE OPEN

TIME OF OBSERVATION	TOTAL LIGHT		DIFFUSE LIGHT		RATIO $\frac{\text{DIFFUSE}}{\text{TOTAL}}$	
	Open	Shade	Open	Shade	Open	Shade
December 12						
8:15– 8:30 A.M.	4.2	2.5	2.1	1.8	0.50	0.72
9:15– 9:30 A.M.	6.3	3.6	3.2	2.3	0.51	0.64
10:00–10:15 A.M.	7.6	5.1	2.9	3.0	0.38	0.59
11:00–11:15 A.M.	10.0	6.8	2.7	2.4	0.27	0.35
12:30– 1:00 P.M.	9.6	7.3	2.9	2.4	0.30	0.33
1:30– 2:00 P.M.	6.2	4.3	2.3	2.1	0.37	0.49
2:30– 3:00 P.M.	4.3	3.1	1.8	2.2	0.42	0.71
3:30– 4:00 P.M.	2.5	2.0	1.3	1.4	0.52	0.70
4:45– 5:15 P.M.	0.9	0.2	0.4	.....	0.44	.....
December 23						
7:00 A.M.	.....	.....	1.6	0.9	.....	.....
8.00 A.M.	.....	.....	4.8	3.3	.....	.....
9:00 A.M.	.....	.....	7.9	5.6	.....	.....
10:00 A.M.	.....	.....	6.7	4.1	.....	.....
11:00 A.M.	.....	.....	7.6	5.8	.....	.....
12:00 M.	.....	.....	4.8	3.8	.....	.....
1:00 P.M.	.....	.....	10.0	6.5	.....	.....
2:00 P.M.	.....	.....	8.1	6.1	.....	.....
3:00 P.M.	.....	.....	7.4	4.9	.....	.....
4:00 P.M.	.....	.....	3.6	2.4	.....	.....
5:00 P.M.	.....	.....	0.6	0.5	.....	.....
January 25						
7:00 A.M.	0.3	0.3	.....	.....	.....	.....
8:00 A.M.	2.3	1.5	.....	.....	.....	.....
9:00 A.M.	5.7	2.9	2.4	1.8	0.42	0.62
10:00 A.M.	6.8	3.2	2.0	1.8	0.29	0.56
11:00 A.M.	8.7	4.3	3.0	2.6	0.34	0.60
12:00 M.	10.0	5.7	2.9	2.5	0.29	0.44
1:00 P.M.	8.2	5.1	2.7	2.4	0.33	0.47
2:00 P.M.	5.8	3.7	2.0	1.8	0.34	0.49
3:00 P.M.	3.6	2.5	1.6	1.4	0.44	0.56
4:00 P.M.	2.8	1.1	1.0	.....	0.36	.....

out, but the diffuse light showed very little difference in the two stations on those days. On December 23, however, when there was no bright sun, the total light in the tent (all diffuse) was reduced by about one-third. On the bright days the ratio of diffuse light to total light was much higher in the tent than in the open. The

effect of the cheese-cloth tent, therefore, appears to be not only to reduce the total amount of light available to the plants, but also to transform a large proportion of the direct light into diffuse light. The plants growing within the tent have available for photosynthesis less total light than the plants growing outside, but a larger proportion of this light is diffuse.

TEMPERATURE

The temperatures in each of the two stations were recorded by Fries thermographs placed in shelters so constructed, with double roof and open sides, as to protect the instruments from the direct rays of the sun and from rain without hindering the free circulation of air. The thermographs had been carefully adjusted during a few weeks' trial previous to the beginning of the experiments. After having been placed in the field, they were daily compared with standard thermometers whose bulbs hung near the bellows of the instruments. The thermographs agreed very closely with the thermometers throughout the experiment, and required very little further adjustment.

The average daily temperatures during the course of the experiment were obtained by integrating the records for each day with a planimeter. The results thus obtained, together with the differences between the daily average in the tent and in the open, are given in table III. The records are given in Fahrenheit degrees since the instruments recorded in that scale, but the values have also been calculated in Centigrade degrees, which are given in the last two columns.

Table III shows that there was no marked difference between the temperature within the tent and that outside. The difference was usually less than one degree. Moreover, it was sometimes positive and sometimes negative. The sum of the differences for the total period is only 7°.67 F. The average daily excess of the temperature outside the tent over that inside was therefore approximately 0°.14. This is contrary to the results of STEWART,[5] who found that in the Connecticut Valley the average daily temperature

[5] STEWART, J. B., The effects of shading on soil conditions. U.S. Dept. Agric., Bureau of Soils, Bull. 39. 1907.

TABLE III

AVERAGE DAILY TEMPERATURE

FOR 24 HOURS ENDING AT 4 P.M.	MEAN TEMPERATURE (FAHRENHEIT)		DIFFERENCE	MEAN TEMPERATURE (CENTIGRADE)	
	Open	Shade		Open	Shade
December 4	72.23	.....	.....	22.35	.....
" 5	69.54	70.91	+1.37	20.86	21.62
" 6	75.35	76.14	+0.79	24.08	24.52
" 7	74.54	73.93	−0.61	23.63	23.29
" 8	68.52	69.03	+0.51	20.29	20.57
" 9	71.67	71.12	−0.55	22.04	21.73
" 10	69.68	68.77	−0.91	20.93	20.43
" 11	64.86	61.90	−2.96	18.26	16.61
" 12	62.86	63.56	+0.70	17.14	17.53
" 13	67.03	66.55	−0.48	19.46	19.19
" 14	64.46	64.86	+0.40	18.03	18.25
" 15	67.02	68.13	+1.11	19.46	20.07
" 16	66.85	67.74	+0.89	19.36	19.85
" 17	67.26	68.53	+1.27	19.59	20.29
" 18	64.68	67.40	+2.72	18.16	19.67
" 19	69.00	69.82	+0.82	20.56	21.01
" 20	71.17	72.05	+0.88	21.76	22.25
" 21	67.75	67.60	−0.15	19.86	19.78
" 22	68.90	70.55	+1.65	20.50	21.42
" 23	67.06	67.43	+0.37	19.48	19.68
" 24	65.04	65.10	+0.06	18.36	18.39
" 25	65.34	64.95	−0.39	18.52	18.30
" 26	63.42	63.54	+0.12	17.46	17.52
" 27	58.30	58.45	+0.15	14.61	14.69
" 28	65.76	65.25	−0.51	18.76	18.47
" 29	67.94	67.62	−0.32	19.97	19.79
" 30	72.85	70.51	−2.34	22.69	21.39
" 31	72.00	70.86	−1.14	22.22	21.59
January 1	69.68	68.04	−1.64	20.93	20.02
" 2	68.23	68.01	−0.22	20.13	20.01
" 3	72.13	71.02	−1.11	22.29	21.68
" 4	73.81	72.81	−1.00	23.23	22.67
" 5	72.28	71.78	−0.50	22.38	22.10
" 6	68.62	68.47	−0.15	20.34	20.26
" 7	63.29	62.17	−1.12	17.38	16.76
" 8	62.69	61.39	−1.30	17.05	16.33
" 9	66.67	65.28	−1.39	19.26	18.49
" 10	66.58	66.64	+0.06	19.21	19.24
" 11	65.07	64.80	−0.27	18.37	18.22
" 12	66.89	66.60	−0.29	19.38	19.22
" 13	65.69	65.14	−0.55	18.72	18.41
" 14	71.80	69.51	−2.29	22.11	20.84
" 15	67.50	65.73	−1.77	19.72	18.74
" 16	67.42	67.09	−0.33	19.68	19.49
" 17	65.33	64.13	−1.20	18.52	17.85
" 18	63.84	63.45	−0.39	17.69	17.47
" 19	65.58	67.00	+1.42	18.66	19.44
" 20	66.40	66.37	−0.03	19.11	19.09
" 21	65.96	65.18	−0.78	18.87	17.88

TABLE III—*Continued*

For 24 hours ending at 4 P.M.	Mean temperature (Fahrenheit)		Difference	Mean temperature (Centigrade)	
	Open	Shade		Open	Shade
January 22.....	71.24	70.24	−1.00	21.80	21.24
" 23.....	68.05	68.57	+0.52	20.03	20.32
" 24.....	65.98	66.87	+0.89	18.88	19.37
" 25.....	66.35	66.22	−0.13	19.08	19.01
" 26.....	62.58	64.40	+1.82	16.99	18.00
" 27.....	63.13	64.10	+0.97	17.29	17.83
" 28.....	60.20	60.86	+0.66	15.67	16.03

within a cheese-cloth tent was 1–3° F. higher than that outside. It is possible that the climatic conditions in these two regions are sufficiently different to account for the apparently different effects of the cheese-cloth, but it should be stated that STEWART obtained his average daily temperature from the maximum and minimum temperatures of each day, a method which does not give the true average temperature. It is evident from the data of table III that under the climatic conditions prevailing in Western Cuba during the months of December and January the cheese-cloth tents used for shading tobacco in that region show very little tendency to retain heat.

Relative humidity

The relative humidity in the two stations was recorded by means of Draper hygrometers. These instruments, like the thermographs, had been kept under observation for several weeks previous to the beginning of the experiments, and during that time had been adjusted to correspond through the lower part of their range with a sling psychrometer. Since the range of these instruments was not adjustable, it was not possible to make them correspond with the psychrometer readings through the whole range of the scale. At night, when the sling psychrometer regularly showed a relative humidity of 100 per cent, the hygrometers still showed a deficit of 4–5 per cent. The recording instruments, however, agreed with each other throughout the range of the movement of the pens. Each week during the course of the experiment the instruments were compared by being placed side by side for one hour. They

TABLE IV

AVERAGE DAILY HUMIDITY

FOR 24 HOURS ENDING 4 P.M.	HYGROMETER			SLING PSYCHROMETER		
	Open	Shade	Difference	Open	Shade	Difference
December 1	79.5	79.0	−0.5	.....	.....	.....
" 2	78.5	79.5	+1.0	.....	.....	.....
" 3	79.5	80.0	+0.5	.....	.....	.....
" 4	79.5	80.0	+0.5	.....	.....	.....
" 5	84.5	84.0	−0.5	.....	.....	.....
" 6	78.5	79.0	+0.5	.....	.....	.....
" 7	78.0	78.5	+0.5	.....	.....	.....
" 8	81.0	82.0	+1.0	.....	.....	.....
" 9	81.5	84.0	+2.5	.....	.....	.....
" 10	83.0	85.5	+2.5	86.0	83.6	−2.4
" 11	74.0	77.0	+3.0	46.2	49.0	+2.8
" 12	79.5	81.5	+2.0	47.4	48.6	+1.2
" 13	89.0	91.0	+2.0	86.0	88.6	+2.6
" 14	79.5	82.5	+3.0	49.6	51.4	+1.8
" 15	79.5	83.0	+3.5	51.6	57.4	+5.8
" 16	84.5	86.5	+2.0	59.4	61.6	+2.2
" 17	79.5	81.5	+2.0	53.0	56.8	+3.8
" 18	76.0	77.0	+1.0	46.0	46.6	+0.6
" 19	75.0	78.0	+3.0	44.6	44.8	+0.2
" 20	78.5	78.5	.....	49.0	49.0	.....
" 21	80.0	80.5	+0.5	56.2	54.0	−2.2
" 22	86.0	84.5	−1.5	51.2	53.2	+2.0
" 23	85.5	86.5	+1.0	73.8	73.4	−0.4
" 24	77.5	78.5	+1.0	46.4	47.6	+1.2
" 25	82.5	83.0	+0.5	63.4	63.0	−0.4
" 26	78.0	80.0	+2.0	56.8	61.4	+4.6
" 27	78.0	79.5	+1.5	50.6	52.2	+1.6
" 28	71.0	73.5	+2.5	43.0	44.0	+1.0
" 29	71.0	73.0	+2.0	49.6	52.4	+2.8
" 30	81.0	83.0	+2.0	66.4	70.2	+3.8
" 31	86.0	85.5	−0.5	75.4	77.4	+2.0
January 1	86.5	86.0	−0.5	78.0	79.2	+1.2
" 2	84.0	84.0	.....	62.4	62.4	.....
" 3	82.0	82.0	.....	64.0	68.4	+4.4
" 4	81.5	83.0	+1.5	64.0	63.6	−0.4
" 5	87.5	88.5	+1.0	73.2	73.8	+0.6
" 6	79.5	81.0	+1.5	57.8	57.8	.....
" 7	78.0	80.0	+2.0	61.4	63.4	+2.0
" 8	81.0	83.0	+2.0	65.6	71.2	+5.6
" 9	85.5	87.5	+2.0	78.4	78.8	+0.4
" 10	74.5	79.5	+5.0	47.4	54.0	+6.6
" 11	74.0	77.0	+3.0	45.8	54.4	+8.6
" 12	70.0	74.0	+4.0	43.0	50.2	+7.2
" 13	71.5	77.0	+5.5	42.4	48.4	+6.0
" 14	75.0	78.5	+3.5	55.6	59.6	+4.0
" 15	78.0	83.0	+5.0	65.2	70.6	+5.4
" 16	77.5	81.5	+4.0	58.2	58.4	+0.2
" 17	77.5	80.5	+3.0	60.4	66.2	+5.8
" 18	83.0	86.0	+3.0	67.0	69.4	+2.4
" 19	77.5	82.0	+4.5	46.0	50.2	+4.2
" 20	77.0	80.5	+3.5	53.6	63.2	+9.6

TABLE IV—*Continued*

For 24 hours ending 4 P.M.	Hygrometer			Sling psychrometer		
	Open	Shade	Difference	Open	Shade	Difference
January 21	77.0	80.5	+3.5	50.8	57.8	+7.0
" 22	76.5	80.0	+3.5	48.4	58.2	+9.8
" 23	80.0	83.0	+3.0	57.2	66.4	+9.2
" 24	78.5	82.5	+4.0	58.2	62.8	+4.6
" 25	76.0	80.0	+4.0	50.2	54.4	+4.2
" 26	77.0	80.5	+3.5	52.0	60.0	+8.0
" 27	77.0	80.0	+3.0	53.8	56.8	+3.0
" 28	74.0	78.5	+4.5	50.0	54.8	+4.8

were readjusted only once during the entire time. To overcome the effects of accidental differences not detected the hygrometers of the two stations were interchanged each week. From what has been said it will be clear that only a relative value can be ascribed to the humidity records. Since the two instruments agreed with each other when kept under the same conditions, this record, nevertheless, ought to give a fair idea of the differences in relative humidity inside and outside the tent. The daily averages were obtained by integrating the curves of the records (which are on circular charts) by means of a Bristol-Durand radii-averaging instrument. These averages, together with the differences between the figures for the two stations for each day, are given in table IV. In addition to the hygrometer records, daily observations were taken at noon in the two stations by means of a sling psychrometer. The results of these observations are given in the fourth and fifth columns of the table, where each figure represents the average of five readings. These observations taken at noon represent approximately the lowest relative humidity for each day.

Table IV shows that the relative humidity is higher inside than outside the tent, but the difference is not as great as that found by STEWART in Connecticut. Owing to the greater quantities of water given off by the plants with increase in leaf surface during growth, the difference is greater toward the end of the season than at the beginning. This relation is brought out still more strikingly by the differences during the day, when the plants are actively transpiring. The difference in the relative humidity inside and

outside the tent at noon is much greater than the difference in the average relative humidity of the two stations.

Evaporation

Records of the relative rates of evaporation in the two stations were obtained by means of the porous cup atmometer described by LIVINGSTON[6] and since frequently used in studies dealing with the relation between plant functions and meteorological conditions.

TABLE V

EVAPORATION FROM POROUS CUP ATMOMETERS

Date	Open	Shade	Date	Open	Shade
December 2	19.9 cc.	15.5 cc.	December 31	9.7 cc.	4.2 cc.
" 3	16.4	12.4	January 1	8.1	4.3
" 4	10.8	10.0	" 2	12.6	6.5
" 5	12.7	9.2	" 3	12.2	7.8
" 6	22.5	16.2	" 4	12.0	6.4
" 7	18.2	12.8	" 5	5.5	1.8
" 8	15.6	12.0	" 6	13.6	8.3
" 9	14.4	10.6	" 7	14.1	6.8
" 10	15.6	11.3	" 8	12.5	6.0
" 11	22.3	16.4	" 9	6.2	2.4
" 12	17.0	12.6	" 10	18.5	8.5
" 13	1.4	0.0	" 11	19.8	8.5
" 14	14.8	10.7	" 12	22.7	10.0
" 15	18.3	13.5	" 13	19.4	8.1
" 16	14.1	10.8	" 14	21.3	9.3
" 17	21.0	15.2	" 15	15.5	6.2
" 18	25.2	16.7	" 16	13.8	5.9
" 19	22.1	16.0	" 17	14.8	5.7
" 20	22.1	15.7	" 18	6.8	1.7
" 21	16.6	12.1	" 19	13.6	5.4
" 22	13.8	9.5	" 20	14.4	5.7
" 23	11.7	7.0	" 21	14.6	6.1
" 24	20.2	13.3	" 22	15.8	7.1
" 25	16.3	11.1	" 23	13.8	6.1
" 26	20.7	12.3	" 24	12.8	6.0
" 27	21.1	13.3	" 25	15.3	7.4
" 28	30.3	18.3	" 26	13.8	6.6
" 29	16.4	16.8	" 27	13.8	6.2
" 30	13.0	8.0	" 28	14.4	6.6
Total				905.9	540.9

The instruments were set up in such a manner that the cups were about one meter above the ground, the water being supplied to the cups by means of burettes. With the growth of the surrounding plants the cups were partly shaded, but this was not considered

[6] LIVINGSTON, B. E., A simple atmometer. Science N.S. **28**:319, 320. 1908; see also Plant World **15**:157–162. 1912.

disadvantageous, since the cups were thus probably exposed to nearly the same average conditions of light and shade as the leaves. The record of evaporation from the cups during the course of the experiments is given in table V. The figures are all reduced to terms of evaporation from a standard atmometer whose coefficient is taken as unity. They are directly comparable, therefore, with each other and with the figures given by LIVINGSTON[7] and by CALDWELL.[8]

Table V shows that the rate of evaporation is constantly less under the cheese-cloth cover than it is in the open. As with the relative humidity, the difference between the two stations increases with the development of the plants. During the early part of the period covered by the experiments, the cups in the open lost one-fourth to one-third more water than those in the shade, but later the loss from the cups in the open was more than twice that from those in the shade. The increasing divergence of the rates of evaporation corresponds with the increasing quantity of water vapor given off by the growing plants.

Rainfall

As a rule, there is very little rainfall in Western Cuba in the winter months, during which the tobacco crop is grown, consequently tobacco and other crops grown during that season require irrigation. The season during which this experiment was conducted was no exception. The only rather heavy rains occurred on December 13 and December 15, and on January 5. The complete record of rainfall taken from the weather observations at the station during the time of the experiment is as follows:

December	2.....	3.30	mm.	January	1.....	1.02	mm.
"	8.....	3.81	"	"	2.....	0.76	"
"	13.....	11.68	"	"	4.....	1.02	"
"	15.....	7.37	"	"	5.....	12.58	"
"	30.....	1.78	"	"	8.....	1.52	"
"	31.....	1.02	"	"	19.....	0.51	"

[7] LIVINGSTON, B. E., A study of the relation between summer evaporation intensity and centers of plant distribution in the United States. Plant World 14:205–222. 1911.

[8] CALDWELL, J. S., The relation of environmental conditions to the phenomena of permanent wilting in plants. Physiol. Researches 1:1–56. 1913.

Summary of observations on environment

The data relating to the changes in the environment induced by the cheese-cloth shade may be briefly summed up here.

The light intensity is greatly modified by the cheese-cloth shade. The total light under the cloth is reduced by about one-third, but the diffuse light inside the tent is only slightly reduced. It appears, therefore, that the plants inside the tent receive a smaller quantity of total light than the plants outside, but an almost equal amount of diffuse light.

The cheese-cloth tent shows very little effect on the temperature. On the whole, the temperature outside the tent is slightly higher than that inside. The average difference for the 60 days, however, is only about 0°.14 F. This can have but little effect on the transpiration of the plants. It appears that any tendency of the tent to retain heat is compensated by the reduced amount of radiant energy which passes into the tent.

The difference in the relative humidity of the two stations is much greater than the difference in temperature. This difference is restricted to the hours of sunlight, for at night the relative humidity in both stations reaches 100 per cent. During the day the difference is enhanced by the partial retention by the tent of the water transpired by the plant.

The rate of physical evaporation is greater in the open than under the cheese-cloth shade. The divergence of the rates of evaporation in the two stations increases with the development of the plants and the consequent increase in relative humidity under the cheese-cloth.

Aside from the lessening of the illumination and the increase in relative humidity, the cheese-cloth effects a reduction of air currents. All these changes tend to diminish transpiration.

Materials and methods of experimentation

The plants used in this work were grown from seed obtained during the previous season from a single self-fertilized mother-plant, whose offspring was shown by subsequent cultivation both in Cuba and in the United States to be of a pure strain.[9] From a

[9] The strain used was the no. 7 described in the following paper: HASSELBRING, H., Types of Cuban tobacco. BOT. GAZ. **53**:113–126. *pls. 4–10.* 1912.

large number of seedlings, 12 were selected as uniform as possible. The experimental plants were grown in cylindrical galvanized iron tanks 38 cm. high and 30.5 cm. in diameter. These tanks resembled in general construction those described by FORTIER.[10] Each tank was provided with an inlet tube 1.3 cm. in diameter, which ran down the inside of the tank to the center of the bottom where it ended. The upper end of the tube was closed by a screw cap. Each tank was further provided at the rim with two lugs into which hooks could be inserted to facilitate lifting and carrying the tanks. These tanks were fitted into others just large enough to receive them, which were permanently sunk in the ground. To prevent the soil from falling into the space between the walls of the two tanks, the inner tanks were provided near the rim with annular flanges which projected over the rims of the outer tanks. In order to prevent rain water from reaching the soil in the tanks, they were fitted with covers made in two parts, with flanges interlocking in such a way that water could enter only through the opening around the stem of the plant. This was closed as effectually as possible by means of thin sheet rubber. The covers were placed on the tanks every night and during threatening weather.

The soil used for filling the tanks was taken from a well-tilled field which in former years had been used for growing tobacco and other crops. A quantity, somewhat more than sufficient for filling the tanks, was placed on the concrete floor of a closed shed, where it was thoroughly worked over many times, with the addition of successive small quantities of water until the whole mass was brought into a moist, friable condition. The soil was left in a pile for a day to allow the moisture to become uniformly distributed throughout the mass. On the following day it was again worked over several times and run through a screen preparatory to the filling of the tanks.

Before the tanks were filled, a layer of broken stone was placed on the bottom of each in order to form a sort of reservoir for water and to prevent the closing of the inlet tube by the soil. By means of the stones the tanks were all brought to the same tare. They were then filled with soil which was tamped as uniformly as possible

[10] FORTIER, S., Evaporation losses in irrigation and water requirements of crops. U.S. Dept. Agric., Office of Exp. Stations, Bull. 177. 1907.

to give it the same compactness as the soil of the field. Thirty kilograms of soil were put into each can.

The seedlings which had 3 or 4 small leaves were planted in the tanks on November 27, 1908. At that time each plant was given 500 cc. of water at the surface, and 1000 cc. were added to each tank through the inlet tube. The tanks were left under cover of the shed until the following day (November 28), when all the plants had completely recovered from the effects of transplanting. The tanks were then given another liter of water through the inlet tube and enough more was added to those that required it to bring them all to the same weight.

One set of six tanks with plants, and three without plants that had been treated in every way like those containing plants, was placed in two rows among the plants of the regular crop under the cheese-cloth which covered an area of one hectare (2.471 acres). The other similar set was placed in like manner in an adjoining field of one hectare also planted with tobacco. The sets of experimental plants were thus subjected to the same conditions in their respective environments as the plants of the regular crop.

The general development of the experimental plants was in every way normal, and did not differ from that of those among which they stood. The axillary buds were removed as soon as they appeared, so that the plants grew to a single stem without branches. The terminal buds were not removed, however, as is customary in commercial practice.

The shade plants attained a nearly uniform height of 2.1 meters, while the height of the sun plants, which were a little less uniform, averaged about 1.75 meters. The leaves of the shade plants were much larger and thinner than those of the sun plants and the internodes of the stems were longer.

During the course of the experiment the original seedling leaves, which had increased much in size, withered. These were cut off and dried and later were ground up with the rest of the plants from which they had been taken.

In spite of the tamping of the soil in the tanks, it was found that the soil water receded from the upper layers of soil, which became very dry. Whenever this condition led to incipient wilting, the

proper state of moisture was restored by adding measured quantities of water at the surface, the controls being treated in the same way. In all, five liters of water were thus added from November 27 to January 14.

From the time that the tanks were placed in the field, the loss of water from the soil and plants was determined by daily weighings. For these weighings a small platform scale specially constructed for the purpose was used. This scale was equipped with agate bearings and with two riders which could be clamped at any point on the beams, one of which was graduated in units of one gram for the smaller rider. The end of the beam was provided with a pointer which indicated the position of exact balance. The scale was sensitive to one gram with the load of 35 kilograms, approximately the weight of the tanks when filled. It was placed permanently on a low solid platform in a shelter to which the tanks could be conveniently brought. The water lost from the soil and plants was replaced each day by the addition of enough water through the inlet tubes to bring the tanks up to the standard weight. While the plants were small, the quantity of water thus added was measured from a burette, but later, when the daily transpiration was large, the bulk of the water required was added by means of graduated flasks marked for pouring, only the final fractions being run in from the burette. The operation was begun at 4:00 P.M. each day and required about two hours for its completion. The quantity of water thus added was recorded as the daily loss by transpiration from the plants and evaporation from the soil.

For obtaining the total transpiration of the plants, the average quantity lost from the three control tanks was subtracted from the total quantity lost from each of the other tanks in the same station, the loss from the controls being regarded as representing the quantity lost from the soil of the tanks containing plants. The difference caused by the partial shading of the soil of the planted tanks had to be disregarded.

The plants were harvested on January 28, at about the time of maturity of the general crop. At that time the leaves were fully grown and the inflorescence was well developed, a few flowers having opened.

The leaves with their decurrent wings were first cut from each plant and weighed immediately. Thereupon, prints for determining the leaf area were made on blue-print paper. The stems, including the inflorescence, were cut off at the surface of the ground, weighed, and cut into small pieces for drying. In order to obtain the roots, the soil was washed out of the tanks with a stream of water. With the aid of a brush the roots were washed free from adhering soil particles. They were then dried by being pressed between towels and absorbent paper, weighed, and cut up for drying.

To obtain the weight of dry substance, the fresh material was dried at 60–70° C. and ground in a drug mill with the observance of such precautions that the material was quantitatively recovered. The leaves, stems, and roots of each plant were ground separately. The air-dry material thus obtained was weighed, and from each lot four samples of approximately 2 grams each were taken. These were dried to a constant weight in a slow current of hydrogen at a pressure of 6 cm. of mercury and a temperature of 78° C.

In order to make a comparison of the transpiration per unit area of leaf surface for the plants in the two stations, the leaf prints made at the time of harvesting were cut out and weighed, and their area was calculated from the relation of their weight to the weight and total area of the original paper. As a basis for calculating the transpiration per unit area of leaf surface, the average quantity of water transpired during the last five days of the experiment was taken. Since the plants had reached the flowering stage, it may be assumed that there was very little change of area of the leaves during this period. The taking of the average daily transpiration obviated to a certain extent peculiarities which might be exhibited by the transpiration of a single day.

Data

In connection with the presentation of the data, attention may again be called to the fact that the plants used in these experiments were the descendants of a single self-fertilized mother plant whose progeny was shown by subsequent cultivation during two generations to be of a pure strain. For this reason more confidence can

be placed in the results than would be possible if the plants had been selected from an indiscriminate mixture. The result of this selection was manifested in the uniform growth of the plants in each of the two stations. The data relating to the total transpiration of the two sets of plants are given in table VI.

TABLE VI

WATER TRANSPIRED BY PLANTS DURING 60 DAYS OF GROWTH

OPEN			SHADE		
No. of plant	Total water transpired	Water transpired per gram of water-free substance produced	No. of plant	Total water transpired	Water transpired per gram of water-free substance produced
1.....	51,256 cc.	245.21 cc.	10.....	41,117 cc.	194.47 cc.
3.....	41,328	245.52	12.....	37,308	187.40
5.....	45,959	239.48	14.....	35,494	192.20
6.....	44,665	237.62	15.....	33,025	191.38
7.....	45,625	246.54	16.....	32,935	176.31
9.....	44,402	235.93	18.....	31,396	180.16
Average	45,539	241.72	Average....	35,212	186.99

Table VI shows that the sun plants transpired on the average 10 liters (about 30 per cent) more water per plant than the shade plants. Although the figures show considerable fluctuation in transpiration among the individuals of each set, yet if the plants of the two sets are compared in the order of magnitude of their transpiration, it will be found that the difference between the members of the different series is practically the same as the difference between the averages for the whole series. Since the average weight of dry matter produced was the same in the two sets of plants, it follows that the series having the higher total transpiration also has the highest transpiration per gram of dry plant substance. This is substantiated by the figures which show that the shade plants transpired 186.99 cc. of water for the production of one gram of plant substance, while the sun plants transpired 241.72 cc. for one gram, or about 30 per cent more than the shade plants. The quantity of water transpired per unit of dry matter produced is remarkably uniform for the plants within each group. A similar close agreement between the quantities of water transpired per unit

of dry matter produced by plants growing in different nutrient solutions has recently led MAZÉ[11] to conclude that under the same aerial conditions the quantity of water transpired per unit of dry matter produced is constant and independent of the nature of the nutritive solutions or of their concentrations or of the state of development of the plant. It is needless to state that this conclusion could hold good only for solutions which contain all the necessary nutrients and which are not otherwise injurious to the growth of plants.[12]

TABLE VII

TRANSPIRATION PER SQ. DM. OF LEAF SURFACE DURING LAST FIVE DAYS OF GROWTH

No. of plant	Leaf area in sq. cm. both surfaces	Total transpiration for last five days in cc.	Transpiration per sq. dm. of leaf surface in cc.	Average hourly transpiration per sq. dm. leaf surface in cc.
OPEN				
1	22,026	10,941	49.67	0.414
3	19,454	10,158	52.22	0.435
5	23,550	11,230	47.69	0.397
6	20,335	10,376	51.02	0.425
7	21,300	10,936	51.34	0.428
9	21,989	9,755	44.36	0.370
Average ...	21,442	10,566	.....	0.412
SHADE				
10	27,896	8,658	31.04	0.259
12	27,461	8,332	30.34	0.253
14	29,026	8,791	30.29	0.252
15	29,116	6,930	23.80	0.198
16	31,163	7,236	23.22	0.194
18	31,867	7,224	22.67	0.189
Average ...	29,442	7,862	.....	0.224

The relative transpiration per unit area of leaf surface is given in table VII. As has been stated, the figures are based on the transpiration of the last five days. This table brings out the relative leaf areas of the plants grown under the two conditions. The

[11] MAZÉ, Sur la relation qui existe entre l'eau évaporée et le poids de matière végétale élaborée par le maïs. Compt. Rend. **156**:720–722. 1913.

[12] That other conditions, such as deficiency or excess of mineral nutrients, may limit production while transpiration continues has been frequently pointed out in agricultural literature: HELLRIEGEL, H., Beiträge Naturwiss. Grundlagen Ackerbaus Braunschweig. pp. 628–635; VON SEELHORST, Jour. Landw. **47**:369–378. 1899.

average leaf area per plant of those grown in the shade was 8000 sq. cm. greater than that of the plants grown in full light. Yet, as we have seen, in spite of this great difference in leaf area, the shade plants used about 10 liters of water less per plant than the sun plants. The hourly transpiration per unit of leaf surface was nearly 84 per cent greater in the plants in the open. The actual hourly transpiration was probably double that given in the tables, since the calculation was based on a 24-hour day, while the plants did not transpire perceptibly during at least 12 hours of that time. Such a change would not, however, alter the relative value of the figures which chiefly concern us here.

A comparison of the rates of transpiration from the leaves during the last five days with the rates of evaporation from the porous cups during the same period of time shows a fairly close relative agreement between transpiration and physical evaporation. The ratio of transpiration in the shade to that in the open is 1:1.8; while the ratio of evaporation from the atmometers in the two stations is 1:2.1.

The data relating to the fresh weight of the plants are given in table VIII.

TABLE VIII

FRESH WEIGHT OF PLANTS

No. of plant	Leaves	Stems	Roots	Total
Open				
1	409	364	246	1019
3	365	335	199	899
5	433	407	233	1073
6	371	388	182	941
7	412	398	197	1007
9	416	389	215	1020
Average	401	380	212	993
Shade				
10	451	508	257	1216
12	443	494	210	1147
14	467	479	193	1139
15	461	466	186	1113
16	488	483	203	1174
18	502	503	180	1185
Average	469	489	205	1162

The average weight of the shade plants was nearly 170 grams greater than that of the sun plants. Moreover, there was no aberrant case. The members within each group were fairly uniform, but all of the shade plants had a greater weight than any of the sun plants. The distribution of the material within the plants is worthy of note. Here also the results, in whatever direction they point, are true as well for a comparison of the individual plants of the respective groups with each other as for a comparison of the general averages. The average weight of the leaves of the shade plants was 68 grams higher than that of the sun plants, while the difference in the stems was still greater, the difference here being over 100 grams in favor of the shade plants. The fresh weight of the root-systems of the two groups of plants was about the same in both. These relations are especially interesting when considered in connection with the weight and distribution of dry matter in the plants as shown in table IX.

TABLE IX

WEIGHT OF WATER-FREE SUBSTANCE OF PLANTS

No. of plant	Leaves	Stems	Roots	Total
OPEN				
1	82.54	73.09	53.40	209.03
3	67.36	59.72	41.25	168.33
5	75.01	69.71	47.19	191.91
6	74.32	70.76	42.89	187.97
7	74.35	67.62	43.09	185.06
9	73.40	71.05	43.75	188.20
Average	74.50	68.66	45.26	188.42
SHADE				
10	71.61	90.36	49.46	211.43
12	69.67	82.92	46.49	199.08
14	66.42	78.08	40.17	184.67
15	59.61	74.22	38.73	172.56
16	71.65	76.19	38.96	186.80
18	62.69	76.32	35.26	174.27
Average	66.94	79.68	41.51	188.14

While the average fresh weight of the shade plants was nearly 170 grams greater than that of the sun plants, the average dry

weight of the two sets was the same, approximately 188 grams. A more marked contrast even is brought out by a comparison of the distribution of material in the different organs of the plants. The weight of dry substance in the roots was practically identical in the two groups. The stems of the shade plants contained 18 per cent more dry matter than those of the sun plants, but the leaves of the sun plants contained 11 per cent more material than the leaves of the shade plants, although the average total area of shade leaves, as shown in table VII, was 37 per cent greater than that of the sun leaves. To recapitulate, the fresh weight of the shade plants was higher than that of the sun plants. This statement applies also to the leaves and stems when the organs are considered separately, but not to the roots, which were about equal in the two sets. The average dry weight of the whole plants and of the roots was the same in the two sets of plants; but the weight of dry material in the leaves was greater for the sun plants, while that of the stems was greater for the shade plants.

These facts show that on the whole the water content of the

TABLE X

PERCENTAGE OF WATER IN LEAVES, STEMS, AND ROOTS

No. of plant	Leaves	Stems	Roots	Total
		OPEN		
1	79.82	79.92	78.29	79.49
3	81.55	82.17	79.27	81.28
5	82.68	82.87	79.75	82.11
6	79.97	81.76	76.43	80.02
7	81.95	83.01	78.13	81.62
9	82.36	81.74	79.65	81.55
Average	81.39	81.91	78.59	81.01
		SHADE		
10	84.12	82.21	80.75	82.61
12	84.27	83.21	77.86	82.64
14	85.78	83.70	79.19	83.79
15	87.07	84.07	79.18	84.50
16	85.32	84.23	80.81	84.09
18	87.51	84.83	80.41	85.29
Average	85.68	83.71	79.70	83.82

shade plants was greater than that of the sun plants.[13] The figures of table X bear out this relationship, not only with regard to the plants as a whole, but also with regard to the individual organs. The complete data are given in that table.

As might be expected, the greatest difference in water content was found in the leaves, whereas the water content of the roots was about equal in the two groups. In the stems, in spite of the fact that the higher fresh weight corresponds with the higher dry weight, the shade plants, nevertheless, contained the greater percentage of water.

General discussion

Various views have been held as to the relation between transpiration and the production of plant substance, or the influence of these processes upon each other. As early as 1850, LAWES[14] expressed the belief that although the whole subject was as yet a problem, a certain relationship existed between evaporation and rapidity of growth, in that the comparative rate of evaporation of water to some extent indicated the comparative activity of the processes of the plants. He was too cautious an investigator, however, to conclude more than that his experiments indicated some definite relationship between the passage of water through the plant and the production of dry matter. A somewhat similar idea was expressed by FITTBOGEN.[15] HELLRIEGEL[16] in discussing this subject pointed out that, although the curves of growth and of transpiration follow the same general course, they are never parallel or coincident. He considered the dry substance produced merely as a convenient empirical basis from which to reckon the water-utilization of plants. As a result of his experiments, which how-

[13] This fact should be taken into consideration in the curing of shade-grown tobacco.

[14] LAWES, J. B., Experimental investigation into the amount of water given off by plants during their growth; especially in relation to the fixation and source of their various constituents. Jour. Hort. Soc. London **5**:38–63. 1850.

[15] FITTBOGEN, J., Altes und Neues aus dem Leben der Gerstenpflanze. Landw. Vers.-Stationen **13**:81–136. 1871.

[16] HELLRIEGEL, H., *op. cit.* pp. 622–623. 1883.

ever are open to criticism, he concluded that transpiration had no effect on the production of plant substance.[17]

A somewhat unusual standpoint was taken by SORAUER,[18] who regarded transpiration not merely as a mechanical process, but as a physiological function in the sense that it depends upon other physiological processes of the plant. According to him, outer factors do not influence transpiration directly, but only through their action on other functions of the plant.

A relation not less intimate, but in its nature the converse of that conceived by SORAUER, is that which KOHL[19] believed to exist between transpiration and assimilation. He described the influence of transpiration upon assimilation essentially as follows: A rapidly transpiring plant receives, by means of the transpiration stream, a greater abundance of mineral nutrients, and is thereby enabled to produce more organic matter than a plant with lower transpiration. It should be said that KOHL'S assumption was based purely on anatomical observations and not on comparative quantitative determinations. Increased transpiration does not necessarily bring about a greater abundance of mineral matter in plants.[20] A close correlation between transpiration and growth has recently been observed also by LIVINGSTON[21] in wheat seedlings during the early stages of their development.

Although in general the conclusion derived from the work of these authors is that transpiration and assimilation are correlated or at most that transpiration has no influence on production, experiments leading to an opposite conclusion are not wanting.

As early as 1869 SCHLOESSING[22] found that a tobacco plant growing under a shaded bell jar produced more dry leaf substance

[17] HELLRIEGEL, H., *op. cit.* pp. 461–501.

[18] SORAUER, P., Der Einfluss der Luftfeuchtigkeit. Bot. Zeit. **36**:1–13, 17–25. 1878; also Studien über Verdunstung. Forschungen Gebiete Agrikultur-Physik. **3**:351–490. 1880.

[19] KOHL, F. G., Die Transpiration der Pflanzen. pp. 90–116. 1886.

[20] HASSELBRING, H., The relation between the transpiration stream and the absorption of salts. BOT. GAZ. **57**:72, 73. 1914. A complete account of this work will appear later.

[21] LIVINGSTON, B. E., Relation of transpiration to growth in wheat. BOT. GAZ. **40**:178–195. 1905.

[22] SCHLOESSING, TH., Végétation comparée du tabac sous gloche et à l'air libre. Ann. Sci. Nat. Bot. V. **10**:366–369. 1869.

than plants grown in the open; but this experiment is open to several objections, not the least of which is that only the leaves were taken into consideration.

TSCHAPLOWITZ,[23] who gave considerable attention to the effect of transpiration on production, found in many of his experiments an increased production of dry substance as the result of decreased transpiration. From a consideration of these results in connection with those of others,[24] who according to him have found that an excessive depression of transpiration results in a lowering of assimilatory activity, he was led to the conclusion that there exists for plants an optimum magnitude of transpiration; and that if the transpiration exceeds, provided the turgor is always maintained, or falls short of the optimum, it is not possible for the plant to reach the maximum production of which it is innately capable. He regards transpiration as essentially a physical process, the magnitude of which may vary within wide limits without seriously disturbing the character of the processes in the plant, although there may be marked effects on the quantitative results of these processes, that is, on the quantity of the assimilatory products formed.

More decisive are the results of WOLLNY,[25] who grew plants of barley, vetch, alfalfa, flax, and potato under conditions giving three degrees of humidity, and found that with an increase in the degree of humidity there was an increase in the production both of the absolute quantity of fresh material and of dry matter. These experiments seem to indicate that a depression of transpiration results in an increase in the assimilatory activity of the plants.

In the experiments reported in this paper, the plants growing in the open transpired about 10 liters per plant or nearly 30 per cent more water than those grown under shade, yet in spite of this differ-

[23] TSCHAPLOWITZ, F., Über den Einfluss der Blattenflächen, des Zuwachses und der Temperature auf die Verdunstung der Pflanzen. Wiener Obst- und Garten-Zeitung **2**:127–132, 169–175, 222–228. 1877; Landw. Vers.-Stat. **23**:74. 1879 (abstract of address without title); Unters. ü. d. Einwirkung Wärme u. d. a. Formen d. Naturkräfte a. d. Vegetations-Erscheinungen. pp. 1–14. Leipzig, 1882; Gibt es ein Transpirations-Optimum? Bot. Zeit. **41**:352–362. 1883; Untersuchungen über die Wirkung klimatischen Faktoren auf das Wachsthum der Kulturpflanzen. Forschungen Gesamt Gebiet Agrikultur-Physik. **9**:117–145. 1886.

[24] **The authorities are not given.**

[25] WOLLNY, W., Untersuchungen über den Einfluss der Luftfeuchtigkeit auf das Wachsthum der Pflanzen. Inaug. Diss. Halle. 1898.

ence in transpiration the total quantity of dry substance produced was the same in both sets of plants. This fact suggests that transpiration in itself, or the mere passage of water through the plant, has no influence on assimilatory activity provided the water supply does not fall below a certain minimum required to maintain the turgor of the cells.[26]

There is another factor, however, to be taken into consideration in the discussion of the effect of transpiration on assimilation in these experiments. This factor is the reduced illumination to which the plants under cheese-cloth were subjected. The work of many investigators[27] has shown that for many plants in northern latitudes light can be considerably reduced without reducing the assimilatory activity.[28] An explanation of this fact is furnished by BLACKMAN and MATTHAEI,[29] who believe that under natural conditions leaf temperature and the partial pressure of carbon dioxide

[26] See footnote 12.

[27] TIMIRIAZEFF, C., The cosmical functions of the green plants. Proc. Roy. Soc. **72**:424–461. *pls.* 3. 1904.

BROWN, H. T., and ESCOMBE, F., Researches on some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and its surroundings. Proc. Roy. Soc. B **76**:29–111. 1905.

BLACKMAN, F. F., Optima and limiting factors. Ann. Botany **19**:281–295. 1905.

LUBIMENKO, W., Production de la substance sèche et de la chlorophylle chez les végétaux supérieurs aux différentes intensités lumineuses. Ann. Sci. Nat. Bot. IX. **7**:321–415. 1908.

COMBES, R., Détermination des intensités lumineuses optima pour les végétaux aux divers stades de développement. Ann. Sci. Nat. Bot. IX. **11**:75–249. 1910.

ROSÉ, E., Énergie assimilatrice chez les plants cultivées saus différents eclairements. Ann. Sci. Nat. Bot. IX. **17**:1–110. 1913.

SHANTZ, H. L., The effects of artificial shading on plant growth in Louisiana. U.S.D.A., Bur. Pl. Industry, Bull. 279. 1913. In this paper the data refer to fresh weights of the plants.

[28] In this connection it is interesting to note that SHANDER attributes the beneficial effects of Bordeaux mixture other than those that can be ascribed to its action as a fungicide to the shading produced by the coating on the leaves. Such beneficial action occurs, however, only during bright weather; during cloudy weather the effect of shading is injurious. SHANDER, R., Über die physiologische Wirkung der Kupferntriolkalkbrühe. Landw. Jahrb. **33**:517–584. 1901; see also EWERT, R., Der wechselseitige Einfluss des Lichtes und der Kupferkalkbrühen auf den Stoffwechsel der Pflanze. Landw. Jahrb. **34**:233–310. *pls.* 3. 1905, and Weitere Studien über die physiologische und fungicide Wirkung der Kupferbrühen bei krautigen Gewächsen und der Johannisbeere. Zeitschr. Pflanzenkrank. **22**:257–285. 1912.

[29] BLACKMAN, F. F., and MATTHAEI, Miss G. L. C., A quantitative study of carbon-dioxide assimilation and leaf temperature in natural illumination. Proc. Roy. Soc. B **76**:402–460. 1905.

function as limiting factors for photosynthesis, while light is usually present in excess.

It is evident from the writer's experiments that the reduction of light did not result in a lowering of the total production of plant substance; nevertheless, the production for equal areas of leaf surface was lower in the shade leaves than in the sun leaves. The reduction in photosynthesis in the shade leaves was compensated by an increase in leaf area, so that total production was not diminished.

Although shading, and the conditions brought about thereby, had no influence on the total elaboration of dry substance, the distribution of dry substance was greatly affected. The distribution of the total dry matter among the various organs of the two sets of plants was as follows:

TABLE XI

	Leaves	Stems	Roots
Sun plants.....	40 per cent	36 per cent	24 per cent
Shade plants.....	36 per cent	42 per cent	22 per cent

The proportion of material deposited in the roots was about the same in the two sets of plants, but the proportion deposited in the leaves was much greater for the sun plants than for the shade plants, although the area of the shade leaves was nearly one-third greater than that of the sun leaves. This condition is in accordance with the general observation that rapidly transpiring leaves are thicker and of firmer structure than leaves developed under conditions of lower transpiration; or, as conversely expressed by SORAUER,[30] of equal weights of fresh leaf substance that portion containing the greater percentage of dry matter transpires the more rapidly. The condition in the stems was the reverse of that in the leaves. In the shade plants the stems contained 42 per cent of the total dry matter of the plant, while in the sun plants only 36 per cent was deposited in the stems. It appears, therefore, that the shading exercises a distinct influence on the deposition of material in the stems and leaves, but that the influence affects the two organs

[30] SORAUER, P., *op. cit.* p. 391.

in an opposite manner; and that little or no influence is exerted on the deposition of material in the roots.

From a practical standpoint the reduced transpiration effected by the cheese-cloth shade is of importance in regions like Western Cuba, where, as has been stated, most of the tobacco is grown with the aid of hand irrigation; but important as is this direct saving of water, it is probably not so significant as the effect of the cheese-cloth shade in reducing the loss of moisture from the soil, and thus increasing the moisture content of the upper layers.[31] The importance of this effect is shown by the investigations of WOLLNY,[32] and of MITSCHERLICH,[33] and particularly by the long series of researches of VON SEELHORST[34] and his co-workers which show that the yield of crop increases with an increase of the degree of saturation at which the soil is maintained; according to MITSCHERLICH, even to complete saturation.

[31] STEWART found in Connecticut that the moisture content of the soil was always higher under the cheese-cloth than in the open ground. STEWART, J. B., *op. cit.* In the writer's experiments the control tanks under the shade lost respectively 3865, 3932, and 3698 cc.; while those in the open lost 4495, 4525, and 4549 cc. of water.

[32] WOLLNY, E., Untersuchungen über den Einfluss der Wachstums-Factoren auf das Productionsvermögen der Kulturpflanzen. Forsch. Agrik.-Physik. **20**:53–109. 1897–1898.

[33] MITSCHERLICH, E. A., Das Wasser als Vegetationsfactor. Landw. Jahrb. **42**:701–717. 1912.

[34] TUCKER, M., und VON SEELHORST, C., Der Einfluss, welchen der Wassergehalt und der Reichtum des Bodens auf die Ausbildung der Wurzeln und der oberirdischen Organe der Haferpflanze ausüben. Jour. Landswirtsch. **46**:52–63. 1908.

VON SEELHORST, C., Über den Wasserverbrauch der Haferpflanze bei verschiedenen Wassergehalt und bei verschiedener Düngung des Bodens. *Ibid.* **47**:369–378. 1899.

———, Neuer Beitrag zur Frage des Einflusses des Wassergehalts des Bodens auf die Entwicklung der Pflanzen. *Ibid.* **48**:165–177. *pls. 2.* 1900.

VON SEELHORST, C., GEORGES, N., und FAHRENHOLZ, F., Einfluss des Wassergehaltes und der Düngung des Bodens auf die Produktion und die Zuzammensetzung von Futterpflanzen, italienisches Raigras u. Klee. *Ibid.* **48**:265–286. 1900.

VON SEELHORST, C., und GEORGES, N., Der Einfluss der Düngung und des Wassergehaltes des Bodens auf den Bau und auf die Zuzammensetzung der Gerstenpflanze resp. des Gerstenkornes. *Ibid.* **48**:325–347. 1900.

VON SEELHORST, C., und FRECKMANN, W., Der Einfluss des Wassergehaltes des Bodens auf die Ernten und die Ausbildung verschiedener Getreide-Varietäten. *Ibid.* **51**:253–269. 1903.

VON SEELHORST, C., Die Bedeutung des Wassers im Leben der Kulturpflanzen. *Ibid.* **59**:259–291. 1911.

Conclusions

Under the climatic conditions of Western Cuba the transpiration of tobacco plants grown in the open ground is nearly 30 per cent greater than the transpiration of plants grown under the cheese-cloth shade commonly used for shading tobacco in that region (fig. 1). The transpiration per unit area of leaf surface is nearly twice as great in the sun plants as in the shade plants.

The shading of tobacco plants by this grade of cheese-cloth does not seem to result in a diminished production of total plant substance by the shaded plants as compared with other like plants not shaded. Since, however, the leaves of the shade-grown plants have a much greater total area than those of plants grown in the open, it is evident that the quantity of plant material elaborated per unit of leaf area is greater in the plants grown in the open.

Although the total production of dry plant substance is not influenced in any marked degree by the cheese-cloth shade, the distribution of this substance is affected in such a manner that in the shade-grown plants relatively less material is deposited in the leaves and more in the stems than in the corresponding organs of the plants grown in full light. No evident influence is exerted on the deposition of material in the roots.

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A PRELIMINARY INQUIRY INTO THE SIGNIFICANCE OF TRACHEID-CALIBER IN CONIFERAE

PERCY GROOM

In connection with an investigation of the structure of the secondary wood of Indian species of *Pinus*, conducted by W. RUSHTON and myself (**2**), it seemed to me that some points of interest might be revealed by an inquiry into the relation between habitat and systematic affinity on the one hand, and, on the other, width of tracheid as measured in the spring zone. Fortunately, a comprehensive list of measurements of the diameters of such tracheids in American Coniferae is given by PENHALLOW (**4**). These, together with the measurements made by RUSHTON on Indian pines, serve as the basis for the succeeding discussion.

In framing conclusions regarding the significance of the statistics, there are a number of points of difficulty that can be removed only by further research or by information supplied by American botanists and foresters. And it is partly in the hope of exciting such research that this tentative inquiry is published. The difficulties are:

1. The caliber of the spring tracheids of a species varies in the same annual ring with height above the ground, and in different annual rings at the same level.

2. The caliber of the spring tracheids also varies in one and the same species according to the habitat in which the individual tree grows. A number of American species of *Pinus* and other conifers show considerable diversity in the habitat (edaphic and climatic) of the one and the same species.

Possibly one of the two causes above mentioned accounts for the discrepancy between PENHALLOW'S measurements and mine in connection with *Pinus glabra*.

3. Information as to the exact climate (rainfall and atmospheric humidity) of various habitats of species is lacking.

4. Information as to the exact edaphic conditions, and particularly as regards amount of moisture and level of the water-table,

are likewise wanting. To select one example. By several botanists *Pinus Jeffreyi* is described as growing on "dry" or "very dry" gravelly slopes. This statement aroused my doubt because the caliber of the tracheids, according to my hypothesis, seemed to speak in an opposite sense. On referring to H. MAYR'S (3) work I found that he states that this gravel must contain a rich supply of water, though the water must not be stagnant.

5. Information as to depth of root is wanting. The lack of this information is especially significant in relation to American species of *Pinus*, which show so strong a tendency for growth on sandy soil. A species described as growing on dry sand may, by virtue of its long roots or a high water-table, be growing in wet sand.

6. Other features besides width of spring tracheid may affect the supply or expenditure of water, as thickness of sap wood, percentage of spring wood, size and structure of leaf, and aggregate surface (including duration) of the leaves of the species.

Despite the possibilities of disturbance by these factors, the evidence clearly points to the view that caliber of spring tracheids of different species varies directly with climatic or available edaphic humidity, and inversely as the conditions tending to induce desiccation. It also varies with the systematic position of the genus or species.

As I was working especially at the structure of the wood of *Pinus* and as this genus offers the largest number of species and the widest range of habitats of any American coniferous genus, the first inquiry concerns it.

Pinus

For the purpose of this discussion, the genus *Pinus* will be divided into two sections. Section I includes the species whose leaves are haploxylic, whose dwarf shoots have a more or less deciduous sheath, and whose wood has non-denticulate ray tracheids and bordered pits on the tangential walls of the outer summer tracheids. Section II includes diploxylic species with a persistent sheath, denticulate ray tracheids, and no universal tangential pitting in the outer summer tracheids. In the succeeding tables the mean diameter of the spring tracheid is the mean between the radial and tangential diameters.

Considering section I of *Pinus*, it is evident that the first six species, characterized by the narrowest spring tracheids, all occupy sites that are both edaphically dry and climatically dry for long

TABLE I

PINUS, SECTION I

	MEAN DIAMETER OF SPRING TRACHEIDS IN μ		HABITAT*
	Atlantic	Pacific or North Mexican	
P. cembroides	.....	24.0	Dry ridges, dry gravel, Arizona, Mexico, at 3500 ft.
P. edulis	.....	24.5	Dry mesas, slopes, dry gravel, Colorado, Texas, New Mexico, up to 9000 ft.
P. Parryana	.....	32.0	Arid mesas and desert slopes; subtropical in California and Lower California.
P. monophylla	.....	33.0	Dry gravelly slopes, Utah to California, Arizona, at 3000–6000 ft.
P. Balfouriana	.....	32.0	Often alpine, 5000–11,000 ft.; dry or rocky ridges and slopes in California.
P. aristata	.....	33.5	Often alpine, up to 12,000 ft.; dry gravelly ridges in California, Nevada, Arizona, and Colorado.
P. albicaulis	.....	35.0	Alpine; 53° N. to British Columbia to southern California, where it reaches 10,000 ft.
P. flexilis	.....	39.0	Sandy-gravelly, sunny places, from British Columbia down to Texas, Arizona, S.E. California; attaining an altitude of 12,000 ft. in Montana; alpine also in Colorado.
P. reflexa	.....	39.5	Cool moist ravines at 6000–8000 ft. in New Mexico and Arizona.
P. Strobus	41.5	.....	Widespread in Canada and particularly the northeast of the United States; in the north on moist sandy or even swampy soil, but in the southern part of its area also on dry gravel.
P. monticola	.....	43.0	British Columbia, southward to California, etc., 2000–10,000 ft.; in cultivation prefers airy, open, rather moist situations.
P. Lambertiana	.....	45.0	Cascade and Coast Ranges in Oregon, southward along the coast to southern California, in rather moist soil with moist air.

*For particulars as to the habitats of the American Coniferae mentioned in this paper, I rely mainly upon the cited works of H. MAYR and C. S. SARGENT.

periods; two of them are also alpine species. The seventh species, *P. albicaulis*, also is alpine, but at least in the north is in a moister climate than are the preceding six, and there is no record that it is on a very pervious soil. The eighth, *P. flexilis*, is less alpine than

P. albicaulis. The ninth, *P. reflexa*, though in the climatically dry region, occupies moist sheltered spots. The remaining three species are in climatically moister regions, and one of them rather belongs to the Atlantic forest flora. The evidence, therefore, favors the view that narrowness of the spring tracheids is linked with special need for economizing water, and is thus encountered in xerophilous species.

But another interpretation is possible. The first six species, having the narrowest tracheids, all show wood of characteristic structure, differing from that of the others in that their ray parenchyma has "piceoid" pitting, and this conforms with characteristic cone structure, which agrees with that of diploxylic species. Thus these species belong to the peculiar subsection PARA-CEMBRA. Again, the first four of the species belong to one group of this subsection, namely PARRYA, while the remaining two belong to the other group BALFOURIA.

The remaining species differ from the first six in structure of medullary rays and of cone (whose scales have a terminal umbo), so that they are members of the subsection CEMBRA. And of them the first two species, with narrowest spring tracheids, belong to the subdivision of CEMBRA known as the group EU-CEMBRA; while the last four, with widest spring tracheids, are included in the other subdivision STROBUS.

Thus, in the whole series of species belonging to section I, diameter of spring tracheid is rigidly linked with systematic position. It is conceivable, therefore, on the one hand, that width of spring tracheid is a systematic or purely morphological character, not an epharmonic one; or, on the other hand, that these groups and subsections are not natural monophyletic groups, but are polyphyletic collections of types whose likenesses are determined by ecological factors. Yet, on the whole, it seems simplest to suppose that the evolution of section I of *Pinus* has been determined by available water supply, and that group PARRYA and group STROBUS represent extreme types, the former the most xerophilous and the latter the most hygrophilous.

The suggestions above given harmonize with the facts relating to East Indian pines. *P. Gerardiana*, belonging to the group PARRYA

of the subsection PARA-CEMBRA, is xerophilous, has narrower spring tracheids than any other East Indian species, the width agrees sufficiently with those of American species, as it is 31.5 μ; that is, it would occupy, from the same point of view, a middle position if ranged among the American species of PARRYA. The other East Indian pine, *P. excelsa*, belonging to section I, is a member of the group STROBUS. Its habitat is not xerophilous, and its spring tracheids are 38.5 μ in diameter (compared with 41.5 μ of *P. Strobus*).

As table II shows, section II of *Pinus* displays no such simple systematic or ecological relations as does section I. This may be due partly to the difficulties mentioned before in appraising the habit and habitat of each species, and in part to defective classification of this large section.

The first three species in table II, having the narrowest spring tracheids (29.5–33 μ), are clearly xerophilous in habitat. One of the two Pacific types, *P. ponderosa* (29.5 μ), is, according to MAYR, the first pine encountered in going west from the prairies. A little farther west, while the narrower (moister) valleys include the moisture-loving Douglas fir and *P. monticola* (43 μ), the broader (drier) valleys are occupied by prairie or by *P. ponderosa*. Still farther west, on sandy soil, Douglas fir vanishes and *P. ponderosa* is accompanied by *P. Murrayana* (34 μ), which also has narrow spring tracheids. Again, according to MAYR, in a more northern Pacific region, where during the vegetative season the prevailing relative atmospheric humidity is 80–63 per cent, Douglas fir exists; but when the humidity sinks to 54 per cent, Douglas fir is replaced by *P. ponderosa*. MAYR ranges Pacific pines found growing in warm temperate regions as follows, according to their demands for moisture, beginning with those demanding most moisture:

Soil moisture: *P. Jeffreyi* (47 μ), *P. Lambertiana* (45 μ), *P. ponderosa* (29.5 μ), *P. Coulteri* (39.5 μ).

Atmospheric moisture: *P. Lambertiana* (45 μ), *P. Jeffreyi* (47 μ), *P. Coulteri* (39.5 μ), *P. ponderosa* (29.5 μ).

Thus, in these pines which replace one another from site to site, the two with widest spring tracheids demand more moisture than the other two. It is worthy of note that the moisture-loving haploxylic *P. Lambertiana* has narrower spring tracheids than the

TABLE II

PINUS, SECTION II

Letters indicating general distribution are as follows: A, Atlantic; P, Pacific; M, North Mexican.

	MEAN DIAMETER OF SPRING TRACHEIDS			DISTRIBUTION, SOIL, ALTITUDE, ETC.
	Pseudo-strobus	Taeda	Pinaster	
1. P. ponderosa	.....	29.5(P)	.....	Dry rocky ridges, dry valleys, rarely in cold swamps; warm temperate; British Columbia to North Mexico (see subsequent comments).
2. P. tuberculata	.....	30(P)	.....	Dry sandy-gravelly slopes and ridges of Sierra Nevada and Coast Mts. from Oregon to California, 2500–5000 ft.; nearly subtropical.
3. P. rigida	.....	33(A)	.....	Dry gravelly uplands, sandy plains, cold deep swamps; Canada to N. Georgia; warm temperate; sometimes with *P. echinata*.
4. P. Murrayana	.....	.....	34(P)	Alaska to S. California and Arizona; on Sierra Nevada at 8000–9000 ft.; often on dry gravel; cool temperate.
5. P. muricata	.....	.....	35.5(P)	Cold peat bogs, barren sandy gravel, sea slopes of Coast Mts. of S. California; subtropical.
6. P. inops	.....	.....	36(A)	Sandy barren soil, dry heights; New York to South Carolina and Indiana; can be grown on the worst types of dry soil (dunes, etc.); warm temperate.
7. P. contorta	.....	.....	37(A)	Sphagnum bogs, sand dunes, exposed rocky sites, near the coast from Alaska to California; warm temperate.
8. P. arizonica	38(M)	.....	.....	Mountains of S. Arizona, Chihuahua, and Sonora; 6000–8000 ft.; warm temperate.
9. P. chihuahuana	.....	38.5(M)	.....	Mountains of New Mexico, Arizona, Chihuahua, and Sonora; 6000–7000 ft.; warm temperate to subtropical.
10. P. Torreyana	38.5(P)	.....	.....	On sand or sandy loam on the coast of California, where it is exposed to the wind, which often causes it to assume a partly prostrate habit; subtropical.
11. P. pungens	.....	.....	39(A)	Dry gravelly slopes of the Alleghany Mts. up to 3000 ft.; warm temperate.

TABLE II—*Continued*

	Mean diameter of spring tracheids			Distribution, soil, altitude, etc.
	Pseudo-strobus	Taeda	Pinaster	
12. P. Coulteri	.....	39.5(P)	.....	Warm dry slopes on the coast ranges of California; 3000–6000 ft.; warm temperate (see subsequent comments).
13. P. sabiniana	.....	39.5(P)	.....	Dry foothills of W. California, among evergreen oaks; subtropical.
14. P. insignis	.....	40(P)	.....	Sandy soil on coast of California; subtropical (see subsequent comments).
15. P. Banksiana	.....	.....	43(A)	Sandy soil to loam, capable of growing on very dry barren soil or even in peat bogs; Canada to Minnesota, etc.; cool temperate.
16. P. resinosa	.....	.....	44(A)	Sandy soil, usually in less dry soil than *P. Banksiana;* widespread in Canada and N.E. United States; warm temperate.
17. P. glabra*	.....	.....	(44)	Moist sand or rather moist forest soil; South Carolina to Florida; subtropical.
18. P. cubensis	.....	44(A)	37(A)	Subtropical and tropical, on land just above flood area; South Carolina, Florida, West Indies, etc.
19. P. serotina	.....	47(A)	.....	"The pond pine," at margin of swamps in wetter soil than *P. cubensis;* N. Carolina to Florida; subtropical.
20. P. Jeffreyi	.....	47(P)	.....	Moist sandy gravel on mountains in California; 6000–8000 ft.; warm temperate (see subsequent comments).
21. P. clausa	.....	.....	48(A)	Barren stretches of sand on higher ground than *P. cubensis;* Alabama to Florida, coast of Gulf of Mexico; subtropical.
22. P. echinata	.....	.....	48(A)	New York to Florida, Louisiana, and Texas; subtropical.
23. P. palustris	.....	49(A)	.....	Sand, often coarse, rarely low wet soil, never in swamps; along the coast from S.E. Virginia along the Gulf of Mexico to Mississippi.
24. P. Taeda	.....	49.5(A)	.....	Low wet clay or sand, less dry and more loamy soil than *P. palustris;* S. Delaware to Florida, Texas, Arkansas, etc.
25. P. tropicalis†	.....	50.8(A)	50.8(A)	Tropical; Cuba, Isle of Pines.

* 44 is my own measurement; 37 that recorded by PENHALLOW.

† As to the synonymy, and consequently the section to which this pine belongs, I am not informed.

diploxylic *P. Jeffreyi* of apparently similar moisture-demanding habits. The other two with narrowest tracheids are both obviously xerophilous as regards edaphic conditions, and one of them is also climatically so in so far as it is a Pacific species.

As a second group of species may be taken those with narrow spring tracheids, between 34 and 37 μ in diameter. All these occupy physically or physiologically dry soils, or where the evidence of this is least obvious, as in *P. Murrayana*, the species often accompanies *P. ponderosa*. Although *P. inops* (36 μ) occasionally is distributed together with two species belonging to the moist subtropical Atlantic region, namely *P. echinata* (48 μ) and *P. Taeda* (49.5 μ), it is in areas less moist than that region, where *P. rigida* (33 μ) is also encountered.

The next two species, *P. arizonica* (38 μ) and *P. chihuahuana* (38.5 μ), mingling on the mountains of dry Mexico, agree closely in diameter of spring tracheids, although the two species belong to different systematic subdivision of section II of *Pinus*. Closely agreeing with them is *P. pungens* (38.5 μ), which belongs to the same systematic group as *P. arizonica* and is xerophilous in distribution on the Pacific Coast.

The fourth group containing four species, with width of spring tracheids between 39 and 40 μ, includes one Atlantic species growing in very pervious soil (gravel) and three Pacific species growing on soils that we may presume are not pervious gravels, for *P. Coulteri* grows on gravelly loam and *P. insignis* occupies sand and is used to fix sand dunes, being apparently uninjured by the salt water flung over its roots by spring tides (MAYR). Comparison between *P. Coulteri* and *P. ponderosa*, which endures greater drought, has been made above.

The next two species, with the width of spring tracheids between 43 and 44 μ, differ from all those previously discussed in being largely cool temperate in distribution; they are northern forms. So far as supply of moisture is concerned, it is difficult to see why the cool temperate *P. Banksiana* (43 μ) should have relatively wide tracheids, as it can grow well on dry barren sand or in peat bogs; and even *P. resinosa* (44 μ) for a time can endure dry sand. It may be that, just as with a higher temperature of climate, trees

require a higher rainfall, so if narrowness of spring tracheids be a form of protection against desiccation, cold temperate pines might be able to afford to have wider tracheids than warm temperate growing in equally dry situations. *P. Banksiana* (43 μ) has narrower tracheids than *P. resinosa* (44 μ), which usually grows in less dry places. These two species are accompanied often by the haploxylic moisture-loving *P. Strobus* (41.5 μ), whose spring tracheids are narrower, just as is the case with the haploxylic *P. Lambertiana* compared with its diploxylic occasional companion, *P. Jeffreyi*.

The remaining species, excepting *P. Jeffreyi*, are subtropical Atlantic, occurring near the coast in a region where there is a heavy rainfall during summer and winter, and the air is moist. It is significant that in this group of pines possessing the widest spring tracheids, these latter agree sufficiently in diameter whether the species belong to the section TAEDA or to PINASTER. First, there are two, one belonging to each section, with a diameter of 44 μ; then there are four belonging to TAEDA, two with the diameter 47 μ, and two with 49 μ and 49.5 μ, respectively. Lastly, there are two belonging to PINASTER, with the diameter of 48 μ. The species that is the most clearly tropical in distribution is the one having the widest spring tracheids; and such is likewise the case in India where the tropical *P. Merkusii* grows on sites receiving, at least periodically, very considerable supplies of moisture.

If again we range the species in accordance with MAYR's grouping as subtropical, warm temperate, cool temperate, and alpine, in order so far as possible to determine temperature as a factor, the following facts come out.

Subtropical.—The five diploxylic species (nos. 2, 5, 10, 13, 14 in table II), characterized by narrow spring tracheids ranging from 30 to 40 μ, are all Pacific species, with or without marked perviousness of soil. The remaining diploxylic species (nos. 17, 18, 19, 21, 23, 24, 25), having wider spring tracheids, are Atlantic species living in a moist climate. The Pacific and Atlantic groups both include representatives of the sections TAEDA and PINASTER.

Warm temperate.—The first six diploxylic species, with the narrowest spring tracheids ranging from 29.5 to 38.5 μ, include two

Pacific species (nos. 1 and 7) and two Atlantic species (nos. 3 and 6), all four of xerophilous habitat; also two Mexican species (nos. 8 and 9) growing in a dry climate and at least sometimes on dry soil. The three species with widest spring tracheids include two Pacific species (nos. 16 and 20), definitely showing larger demands for moisture of soil and air than the other Pacific warm temperate species, and one Atlantic species (*P. resinosa*) that is not edaphically xerophilous as are the other Atlantic species in this warm temperate group. There is not the sharp contrast between the Pacific and Atlantic species that there is in the subtropical group, possibly because the Pacific species are not at so great a disadvantage as regards supplies of moisture, either because they belong to the northern Pacific, which is characteristically moister than the southern (no. 1 partly, and no. 7), or because they grow on mountains where the climate is moist (nos. 12 and 20), while the Atlantic species with warm tracheids are definitely in dry soils.

Cool temperate and alpine species.—Of the two diploxylic species ranged under this heading, *P. Murrayana*, the Pacific species often on dry ground and reaching alpine situations, has narrower spring tracheids (34 μ) than has the other, *P. Banksiana* (43 μ), though this latter can grow on very dry soils.

Summary.—The American species of *Pinus* having the narrowest spring tracheids are all more or less markedly xerophilous in distribution; those with the widest spring tracheids are all subtropical and more or less hygrophilous in distribution. The few East Indian species show similar characters in this respect. In section I of *Pinus*, so far as the measured American and East Indian species are concerned, differences in tracheid width run parallel with distinction in systematic affinity, thus suggesting that the evolution of the different groups of this section has been determined by the available water supply.

Other North American Coniferae

The succeeding table records the width of the spring tracheids as given by PENHALLOW, and the distribution of other American conifers.

Table III shows that evidence in favor of the view that scantiness and abundance of available water supply are respectively

TABLE III

	Width of spring tracheids in μ			Distribution, soil, altitude, etc.
	Atlantic	North Mexican	Pacific	
Torreya—				
T. californica	.....	.....	38	California, 3000–5000 ft., moist soil near streams; subtropical.
T. taxifolia	40	.....	.....	W. Florida, moist soil; moderately warm.
Sequoia—				
S. gigantea	.....	.....	39	Warm temperate.
S. sempervirens	.....	.....	55	Very moist soil and moist air; subtropical.
Chamaecyparis—				
C. Lawsoniana	.....	.....	18	S.W. Oregon and California, moist soil; warm temperate.
C. nutkaensis	.....	.....	25.5	Alaska to British Columbia, Cascade Mts.; adapted to the moistest air and a larger rainfall than *C. Lawsoniana;* warm to cool temperate.
C. thyoides	32	.....	.....	Maine to N. Florida; cold swamps.
Cupressus—				
C. arizonica	.....	25	.....	Arizona, Sonora, Chihuahua, 3000–5000 ft.
C. Macnabiana	.....	.....	30	California, dry hills and low slopes; subtropical.
C. Goveniana	.....	.....	32	California coast up to 3000 ft., hot rocky slopes, often on banks of streams; tropical.
C. macrocarpa	.....	.....	39	California, south of Monterey Bay, exposed constantly to the sea breezes; subtropical.
Juniperus—				
J. sabinoides	.....	17	.....	Texas to Mexico; up to the limits of vegetation on high mountains in Central Mexico.
J. nana	18	.....	.....	Labrador to New York and Utah.
J. californica	.....	.....	18	Sacramento River to Lower California; dry mountain slopes, desert slopes of Tehachapi Mts.; subtropical.
J. occidentalis	.....	.....	18	6000–10,000 ft., dry rocky ridges of Blue Mts., where it is a shrub on dry hot ridges; cool temperate.
J. monosperma	.....	19	.....	Rocky Mts. to Arizona and Mexico, 3500–7000 ft., gravelly slopes.
J. communis	.....	.....	20.5	Greenland to mountains of California and Arizona.
J. pachyphlaea	.....	21	.....	S.W. Texas to desert ranges of Arizona, 4000–6000 ft., arid mountain slopes; subtropical.
J. Sabina	21.5	.....	.....	Nova Scotia to Rocky Mts. and Montana; slopes and river banks.

TABLE III—*Continued*

	Width of spring tracheids in μ			Distribution, soil, altitude, etc.
	Atlantic	North Mexican	Pacific	
Juniperus (*cont.*)—				
J. utahensis	.....	23	.....	Rocky Mts. to Sierra Nevada, up to 8000 ft., arid hills and slopes.
J. virginiana	32	.....	32	New Brunswick to Florida and tropical forests; Atlantic Coast to the prairies (100° W.) north of 54° N.; snowy summits of Rocky Mts., sea coast of British Columbia; rocky, dry, gravelly soil, sterile sand, meadow, moist, or swampy soil; cold to tropical.
Larix—				
L. americana	39.5	.....	.....	Arctic to West Virginia; in the north on well drained uplands, in the south in cold deep swamps; cool temperate.
L. occidentalis	.....	.....	42	British Columbia to Oregon, Montana, and Idaho, 2000–7000 ft.; moist bottom lands and dry mountain slopes; cool temperate.
L. Lyallii	.....	.....	43	Alberta and British Columbia, 7000–8000 ft. near timber line; "alpine."
Picea—				
P. rubra	29.5	.....	.....	Prince Edward Island to N. Carolina; well drained uplands and mountain slopes.
P. Breweriana	.....	.....	33	California and Oregon, 4000–7000 ft.; mountain peaks and ridges, near the timber line; "alpine."
P. alba	33.5	.....	.....	Labrador to New York to Montana; in the southern regions it is a low tree in swamps; it extends far north into permanently icy ground; cool temperate.
P. sitchensis	.....	.....	34	Alaska to California; moist sand or swamp, or wet rocky slopes in the north; solely coastal (within 50 miles of coast); more sensitive to dryness than to cold.
P. nigra	34.5	.....	.....	Alaska to Labrador to Virginia; in the north in well drained bottom lands; in the south in swamps and sphagnum bogs.
P. Engelmanni	.....	.....	35	British Columbia (5000 ft.), Arizona (11,500 ft.), high mountain slopes, well developed in moist canyons, very high up becomes shrubby; cool temperate.
P. pungens	.....	.....	38	Wyoming to Colorado and Utah, 6500–10,000 ft.; banks of streams, moist valleys; cool temperate.

TABLE III—*Continued*

	Width of spring tracheids in μ			Distribution, soil, altitude, etc.
	Atlantic	North Mexican	Pacific	
Abies—				
A. Fraseri	30.5			S.W. Virginia to Tennessee, 4000–6000 ft., on moist slopes.
A. lasiocarpa			33.5	Alaska to Arizona; 4000 ft. in British Columbia; 12,000 ft. in Colorado; "alpine."
A. magnifica			38.5	S. Oregon, 5000–7000 ft.; Sierra Nevada 6000–10,000 ft.; cool temperate.
A. concolor			39	S. Colorado to California and arid regions of New Mexico and Arizona; "moist canyons" of Californian Sierras; warm cool temperate.
A. amabilis			40	British Columbia to Oregon, high mountain slopes, often with *A. nobilis;* cool temperate.
A. balsamea	40			Labrador to mountains of S.W. Virginia; often in low swampy ground or on well drained soil, often with *Picea alba;* cool temperate.
A. nobilis			41	Cascade Mts., Washington to California, 2500–5000 ft., often with *A. amabilis;* warm to cool temperate.
A. bracteata			41.5	California, 3000–6000 ft., moist cool soil.
A. grandis			49.5	Vancouver to California, Idaho, Oregon, Montana; near the coast on moist ground; in the interior on moist slopes; 2500–7000 ft.; warm temperate.

associated with narrowness and wideness of spring tracheids in the different species or genera is provided by *Torreya*, *Chamaecyparis*, *Sequoia*, and *Juniperus;* somewhat favoring the view are *Cupressus* and *Picea;* indifferent in indication from this standpoint are *Abies* and *Larix*.

The enumerated species of *Juniperus* all seem to be actually or potentially xerophilous in habitat, and, with one exception, have very narrow spring tracheids (17–23 μ). The one exception is *J. virginiana*, which has rather narrow spring tracheids, and a remarkable range of distribution as regards climate and soil (it is possible that the specimen of wood measured was derived from a tree growing in a moist site).

Contrasting with *Juniperus* is *Torreya*, in which both species

inhabit moist places and have relatively wide spring tracheids, 38 μ in the Pacific species, and 40 μ in *T. taxifolia* of the moister climate of Western Florida.

Sequoia sempervirens (55 μ), which not only has larger leaves but also grows in a warmer and damper climate and moister soil than *S. gigantea* (39 μ), exceeds all other measures of North American Coniferae in width of spring tracheids.

Of the two Pacific species of *Chamaecyparis*, *C. Lawsoniana* (18 μ) has unexpectedly narrow spring tracheids, for it grows preferably on moist soil. Compared with it, *C. nutkaensis* has wider spring tracheids (25.5 μ) and grows in cooler and much moister regions, where it is limited to places having the moistest of air. Still wider spring tracheids are those of the Atlantic *C. thyoides* (32 μ), though it grows in cold swamps.

Cupressus arizonica (25 μ), living in the driest region (Mexican), is likewise the species with narrowest spring tracheids. The three other species measured are Californian, and the one with narrowest spring tracheids, *C. Macnabiana* (30 μ), appears to be the one of the least moist habitat, for *C. Goveniana* (32 μ) is said to occur often on the banks of streams, while *C. macrocarpa* (39 μ) is constantly in damp sea air. Yet this last species can be used with *Pinus insignis* to fix sand dunes, and its roots can be laved with sea water, so that without further investigation it is impossible to say that *Cupressus*, as a whole, definitely favors the view here propounded.

The American species of *Abies*, whose tracheids have been measured, neither clearly support nor oppose the view. True it is that *A. lasiocarpa* (33.5 μ), the species that is nearly alpine and is described as "alpine," has with one exception the narrowest spring tracheids, but that exception is *A. Fraseri* (30.5 μ), which grows on moist slopes in moist cool air. So far as habitat is concerned, there appears to be no reason why *A. Fraseri* (30 μ) should have much narrower tracheids than the more northern Atlantic form *A. balsamea* (40 μ), which indeed often grows in swamps, and sometimes even sphagnum swamps, unless, indeed, the cause be the same as that already suggested in connection with the parallel cases of *Pinus Banksiana* and *P. resinosa;* apparently *A. balsamea* and *P. Banksiana* are found growing together.

Of the genus *Picea*, *P. rubra* (29.5 μ) is the species having the narrowest spring tracheids. Of the next two species, *P. Breweriana* (33 μ) is the only American Pacific alpine (or subalpine) species; while *P. alba* (33.5 μ) goes very far north, where, according to MAYR, it can grow on permanently icy soil. Farther south *P. alba* often occurs in swamps, including occasionally sphagnum bogs, and frequently mingles with *P. nigra* (34.5 μ). The remaining three species, *P. sitchensis* (34 μ), *P. Engelmanni* (35 μ), and *P. pungens* (38 μ), grow in places where air and soil are moist. Thus the three species whose habitats are regularly or potentially xerophilous have the narrow spring tracheids, whereas the two species with the widest spring tracheids show clear demands for more moisture. Yet this indication is weakened by *P. rubra* and *P. sitchensis*. The three Atlantic species (29.5–34.5 μ) belong to the section MORINDA; of the four Pacific species, *P. Breweriana* (33 μ) is in section OMORICA, while the other three (34–38 μ) belong to CASICTA, and their delicate loose cone scales seem to suggest that moistness of air characterizes their habitat.

Larix shows the narrowest spring tracheids in *L. americana* (39.5 μ), which reaches arctic sites, and, when farther south (reaching Virginia), grows in cold deep swamps, and particularly occurs in sphagnum swamps. Of the other two species, *L. occidentalis* (42 μ) and *L. Lyallii* (43 μ) differ but little in width of spring tracheids, but the one with wider tracheids is that which is nearly alpine, in fact is often termed "alpine." Thus *Larix* gives no clear indication of a correlation between narrowness of tracheid and xerophily of habitat. Compared with the evergreen conifers of the same habitat, the spring tracheids of *Larix* are usually wider, as might be anticipated (1).

In connection with the question of the influence of two of the factors deciding the width of the spring tracheids, namely systematic affinity and habitat, some suggestive results are yielded by a comparison of these widths in different species occupying the same habitats.

In the northern Atlantic region, *Abies Fraseri* (30.5 μ) and *Picea nigra* (34.5 μ) often grow together, as do *Abies balsamea* (40 μ) and *Picea alba* (33.5 μ). These two species of *Picea*, only

slightly differing in tracheid width, often mingle; thus in a second manner is shown that there is some problem as to the unexpected width of spring tracheids in *Abies balsamea*. *Juniperus virginiana* (32 μ) often mingles with the two species of *Picea* in moist ground and does not differ much from them in tracheid width. But *Pinus Strobus* (41.5 μ), often replacing these three species, and *Pinus Banksiana* (43 μ), often occurring with *Picea alba*, though agreeing fairly with one another, have much wider tracheids than these three species. *Larix americana* (39.5 μ) often largely replaces *Abies balsamea* (40 μ) and *Picea alba* (33.5 μ) on sphagnum bogs.

On the warm temperate Pacific coast, *Pinus insignis* (40 μ) and *Cupressus macrocarpa* (39 μ) are often grown together as dune fixers, and nearly agree in tracheid width.

Still closer agreement characterizes *Abies concolor* (39 μ) and *Sequoia gigantea* (39 μ), which are often associated in the warm to cool temperate Pacific forests. Again, the equivalent species of *Pinus* have relatively wide spring tracheids, for *P. Jeffreyi* (47 μ) often is associated with *Abies concolor* (39 μ), whose demands for moisture are about equal to those of *P. Lambertiana* (45 μ) (MAYR).

In the cool temperate Pacific region, the deciduous *Larix occidentalis* (42 μ) has much wider spring tracheids than its frequent companion *Pinus Murrayana* (34 μ); while *Abies nobilis* (41 μ) and *Abies amabilis* (40 μ), which occur together, differ only slightly in width of spring tracheids.

In the Pacific so-called alpine region, there is a remarkable approximate agreement in the width of spring tracheids in three genera: *Abies lasiocarpa* (33.5 μ), *Picea Breweriana* (33 μ), *Pinus Balfouriana* (32 μ), *Pinus aristata* (33.5 μ), and *Pinus albicaulis* (35 μ). All these contrast with the alpine larch, *Larix Lyallii* (43 μ), whose wide tracheids correspond with the deciduous habit.

American deciduous species of Quercus

In a previous paper (1) I have shown that the width of the spring vessels in *Quercus* is decided partly by the habit of the species, and that these vessels are wider in deciduous than in evergreen species, even though the latter may grow in moist Florida, as is the case with *Q. virginiana*. The subjoined statistics suggest strongly

TABLE IV

American deciduous species of Quercus

W, white oaks; B, black oaks; all are warm temperate excepting *Q. lobata*, which is subtropical.

	Width of widest spring vessels				Habitat
	Atlantic		Pacific		
	W	B	W	B	
Q. Garryana	.....	.....	0.338	.....	Dry habitat; Vancouver to California and Oregon; dry gravelly slopes of low hills; also between prairie and pine forests.
Q. lobata	.....	.....	0.356	.....	Subtropical (in the same region as the evergreen *Q. chrysolepis*, *Q. Wislizeni*, and *Q. agrifolia*) in California; often after losing its leaves in dry autumn, acquires fresh ones after rain in November, hence tending to be sub-evergreen.
Q. obtusiloba	0.362	.....	.....	.....	Dry sandy gravel, or on hard impervious loam where dryness and moistness alternate suddenly; Cape Cod to Florida, Mississippi, Texas, etc.
Q. macrocarpa	0.372	.....	.....	.....	Variable as regards soil, but showing power of enduring some degree of dryness by growing on higher sites at the edge of prairie or on dry hills in northwest region of its area, which is wide, extending from Nova Scotia and Ontario to Minnesota, Texas, etc.
Q. Macdonaldi	.....	.....	0.372	.....	Islands off California.
Q. californica	.....	.....	.....	0.378	W. Oregon, coast ranges of California, 7000–8000 ft. in western slopes of Cascade Mts., to mountains of S. California (optimum 6000 ft.).
Q. Prinus	0.387	.....	.....	.....	Various habitats; Ontario to Alabama, Tennessee, etc.
Q. rubra	.....	0.387	.....	.....	No special soil; goes farther north than any other American oak, and on Alleghany Mts. ascends into the fir region.
Q. coccinea	.....	0.412	.....	.....	Usually light sand, but also dry gravelly uplands and prairies; Ontario to Alleghany Mts., and North Carolina to Nebraska, etc.
Q. tinctoria	.....	0.412	.....	.....	Dry gravelly uplands and ridges; Ontario to Minnesota, Florida, Mississippi, Texas, etc.
Q. palustris	.....	0.419	.....	.....	Good wet soil (not swamp) at the edges of swamps and margins of rivers; Canada to Missouri, Virginia, Arkansas, etc.

TABLE IV—*Continued*

	Width of widest spring vessels				Habitat
	Atlantic		Pacific		
	W	B	W	B	
Q. aquatica	.....	0.438	.....	.....	Sandy borders of swamps; Delaware to Florida, Gulf States, Texas, Oklahoma, etc.
Q. Muhlenbergii	0.444	.....	.....	.....	Variable habitat; dry hills to deep rich bottom lands and rocky banks of streams; Ontario to Columbia, N. Louisiana, Texas, etc.
Q. alba	0.45	.....	.....	.....	Optimum soil is fresh (rather moist) loam in undulating country or on the banks of streams; but the habitat varies from sandy plains to gravelly ridges; Ontario to N. Florida, Minnesota, Texas, etc.
Q. bicolor	0.45	.....	.....	.....	Borders of streams and swamps; Ontario to N. Georgia and W. Missouri; does not extend so far south as *Q. alba*.
Q. Michauxii	0.587	.....	.....	.....	Borders of swamps and streams; Delaware to Florida, Gulf States, Kentucky, etc.

that in the deciduous species width of spring vessel is at least largely determined by systematic affinity and by available water supply; the spring vessels being narrower in species belonging to physically or physiologically drier places, and for the same type of habitat being narrower in black oaks than in white oaks.

The points of significance in connection with the deciduous oaks are:

1. Of the six species with narrowest spring vessels (0.338–0.378 μ), four are Pacific species and characterized by drier climate than the Atlantic species. Among the four Pacific species, the one with the narrowest vessels is the one whose soil is definitely stated to be dry gravel. The second is subtropical in the region of evergreen species, and itself approaches the evergreen stage. Of the other two, one has a maritime climate, and the other no dryness of habitat (apart from the Pacific climate). Of the two Atlantic species, one occupies an edaphically dry habitat, while the other, though variable as regards habitat, shows a power of living on at least rather dry sites.

2. Considering the Atlantic species, and taking separately the two series representing respectively the white oaks and black oaks, the former of these begins with the two species just referred to, continues with two species of variable habitat as regards soil, passes to one in which the optimum soil is rather moist but varies, and concludes with two species confined to thoroughly moist soil. The last of these also occurs in the region where the American pines exhibit the greatest caliber of tracheids, namely, Florida and the Gulf States.

The series of Atlantic black oaks commences with wider vessels than the series of white oaks. The first species shows no special choice of soil, but can grow farther north than any other American warm temperate species; the next two species clearly show preference for dry situations, or at least a capacity for thriving on dry gravels; the series, like that of the Atlantic white oaks, concludes with two species confined to thoroughly moist soil on the borders of swamps and rivers.

Summary

1. There is considerable evidence that the width of the spring tracheids in evergreen Coniferae is largely decided by two factors, systematic affinity and available water supply. So far as the latter is concerned, the spring tracheids are generally narrowest in species of xerophilous habitat.

2. In American species of *Pinus* belonging to section I (Haploxylon), variation in the width of the spring tracheids runs quite parallel with difference of systematic affinity and of available water supply (including influences promoting transpiration). Thus the first step in the evolution of this section of *Pinus* would appear to have been a division into a more xerophilous type (ancestral PARACEMBRA), and a less xerophilous type (ancestral CEMBRA), and each of these subsections would appear to have undergone similar division into more or less xerophilous groups, that is, into PARRYA and BALFOURIA, also EU-CEMBRA and STROBUS. The two East Indian species, *P. Gerardiana* and *P. excelsa*, structurally accord with this theory.

3. Among American species of *Pinus* belonging to section II (Diploxylon), those with narrow spring tracheids are more xerophi-

lous in distribution, while those with the widest tracheids belong to a subtropical or tropical moist climate. Though in general this section of *Pinus* supports the theory given in paragraph 1, there are in it certain species in which width of tracheid does not appear to correspond with the supply of available water. Such discrepancies, whether real or only apparent, may be due to one or more of the intervening causes mentioned in paragraph 6.

4. Species of other North American genera of evergreen Coniferae show differences in the width of spring tracheids that may possibly be partly due to differences in affinity; as species of the same habitat, but belonging to different genera, may differ considerably in tracheid width, or, on the other hand, may approximate to agreement. Some of these genera, namely, *Torreya*, *Chamaecyparis*, *Sequoia*, and *Juniperus*, support the view that the width of the spring tracheid is correlated with available water supply; somewhat favoring the view are *Cupressus* and *Picea;* indifferent in indication are *Abies* and *Larix*.

5. The theory here propounded derives support from measurements of the width of the spring vessels of American deciduous species of *Quercus*. For narrowness and wideness of spring vessels in the main are respectively associated with scantiness and abundance of water supply. But in the same kind of habitat the deciduous black oaks would seem to have narrower spring vessels than are possessed by the deciduous white oaks.

6. Though the evidence as a whole strongly favors the theory here propounded, much fuller information is necessary before a safe conclusion may be drawn. Hence this inquiry and the suggestions here given must be regarded as tentative and issued in the hope of stimulating inquiry in regard to factors that may intervene, such for instance as the following: climate (including evaporation power), exact soil water-content, level of water-table, etc., that form the environment of the different species of conifers; also, depth of root, duration of foliage and size of aggregate leaf surface, rate of transpiration, width of sap wood, etc., in the different species; also, variations within one and the same species in regard to the features just mentioned, as well as in the width of the spring tracheids, in different habitats.

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NOTE ON THE ASCOSPORIC CONDITION OF THE GENUS ASCHERSONIA MONTAGNE[1]

ROLAND THAXTER

(WITH SEVEN FIGURES)

The genus *Aschersonia* includes a group of entomogenous fungi which have hitherto found a place among the Sphaeropsideae, since, as far as I am aware, no ascosporic condition has as yet been observed in connection with any of them. Although a great majority of the forty or more species which have been described, for the most part from the tropics, are said to occur on the leaves, etc., of various hosts among the vascular plants, there can be no question in the mind of anyone who has had an opportunity to examine them in a fresh condition that they are strictly entomogenous, like the species of the genus *Hypocrella*, which have a similar habitat on various types of scale insects; and, as is well known, two species of the genus have been successfully employed in Florida against certain scales attacking *Citrus*. As in the case of other entomogenous fungi, the character of the host plant on which species of *Aschersonia* have been reported is thus a matter of very little importance, except in so far as it may suggest the nature of the scale which has been attacked while feeding on it. Although it is not improbable that some of the species are not restricted to closely similar hosts, there are indications that others are more definitely conditioned in this respect, and an examination of this matter from the entomological side is much to be desired. Unfortunately, very little information is available in this connection, and the actual hosts of the too numerous and ill defined species, a majority of which have been described within the past fifteen years, are, with a very few exceptions, quite unknown.

In view of the general characteristics of *Aschersonia*, it has been naturally assumed that the ascosporic form, if it exists, would find a place among the Hypocreaceae, the often bright though very variable and inconstant colors and comparatively soft con-

[1] Contributions from the Cryptogamic Laboratories of Harvard University, LXII.

sistency of the different species pointing to this conclusion. It has been suggested that they might be imperfect conditions of species of the ascomycetous genus *Hypocrella*, with which, owing to their similar mode of life, they are apt to be associated; and this suggestion is still further supported by the fact that when a flat hemispherical type of *Aschersonia* has become blackened by age and exposure, or colored by the sooty disintegrated material of accompanying *Capnodia*, which grow on the excreta of various hemipterous insects, it is often difficult to distinguish the two by their gross appearance. Definite information in regard to this connection, however, has hitherto been lacking, and, as already mentioned, I have found no record of observations which might throw light upon it. P. HENNINGS in the ASCHERSON *Festschrift*, where he discusses the validity of the generic name and certain other matters, states that he was informed by ZIMMERMAN, whose contributions to the knowledge of Javan entomogenous fungi are well known, that although he sought for them with care he never encountered any individuals which showed indications of an ascosporic fructification.

During the past year (1912–1913) I had an opportunity to spend some months on the islands of Grenada and Trinidad, and having the matter in mind made a special effort to discover this problematical ascosporic form. In the locality where I remained during practically my whole stay in Grenada, *Aschersoniae* were by no means numerous, and only three species were met with. These forms, moreover, were comparatively rare, although one of them, the well known and characteristic though very variable *A. turbinata*, was found several times. A few specimens of this species in the original gathering from a certain locality showed, when carefully examined, certain not very conspicuous but suspicious looking pustules, containing cavities unlike those of the pycnidia, which appeared to be young perithecia, and by a systematic search in the same spot I was able to obtain numerous specimens bearing the perfect form fully and characteristically developed. Unluckily, the majority of these specimens were accidentally destroyed by fire, together with many other mycologic treasures, but a sufficient number were saved, both dry and in alcohol.

In Trinidad, where the flora as well as the insect fauna is far more varied, *Aschersoniae* were numerous, and in most instances it was possible to gather abundant material of each species. These gatherings in the case of four or five species usually included the ascosporic condition, which was often abundant, and, as in the case of *A. turbinata*, occurred either by itself or associated on the same stroma with the pycnidial form; so that there could be no question as to the actual connection of the two conditions. As far as could be determined, the position of growth, whether on the upper or lower side of a leaf for example, in shady and moist or in drier and more open situations, has little if any influence on the development of the perfect condition. In some instances it appeared to follow the pycnidia in older specimens, while in others it was as evidently primary in its development and unaccompanied by pycnidia.

The general character and appearance of the perfect condition recall those of some species of the genus *Cordyceps*, to which *Aschersonia* is evidently closely related; and, as in this instance, the association of the perithecia and the development of perithecial stromata varies in different cases. In some instances the perithecia may be closely and definitely grouped in a compact and prominent stromatic outgrowth from the general stroma, which may be otherwise sterile, while in many the whole stromatic mass may become transformed into a pulvinate aggregation of densely crowded perithecia. The general appearance of such forms, which in one instance may be definitely stalked, is not unlike that of some species of *Cordyceps* or *Hypomyces*. In other cases the perithecia may be irregularly scattered in a somewhat looser stroma, and might at first be mistaken for the common *Cordyceps* (*Torrubiella*) *arachnophila*, which is often found on leaves with or without its imperfect or *Isaria* (*Gibellula*) condition. But in this instance, although the host may be as completely obliterated as it is by *Aschersoniae*, the perithecia are always much more prominent.

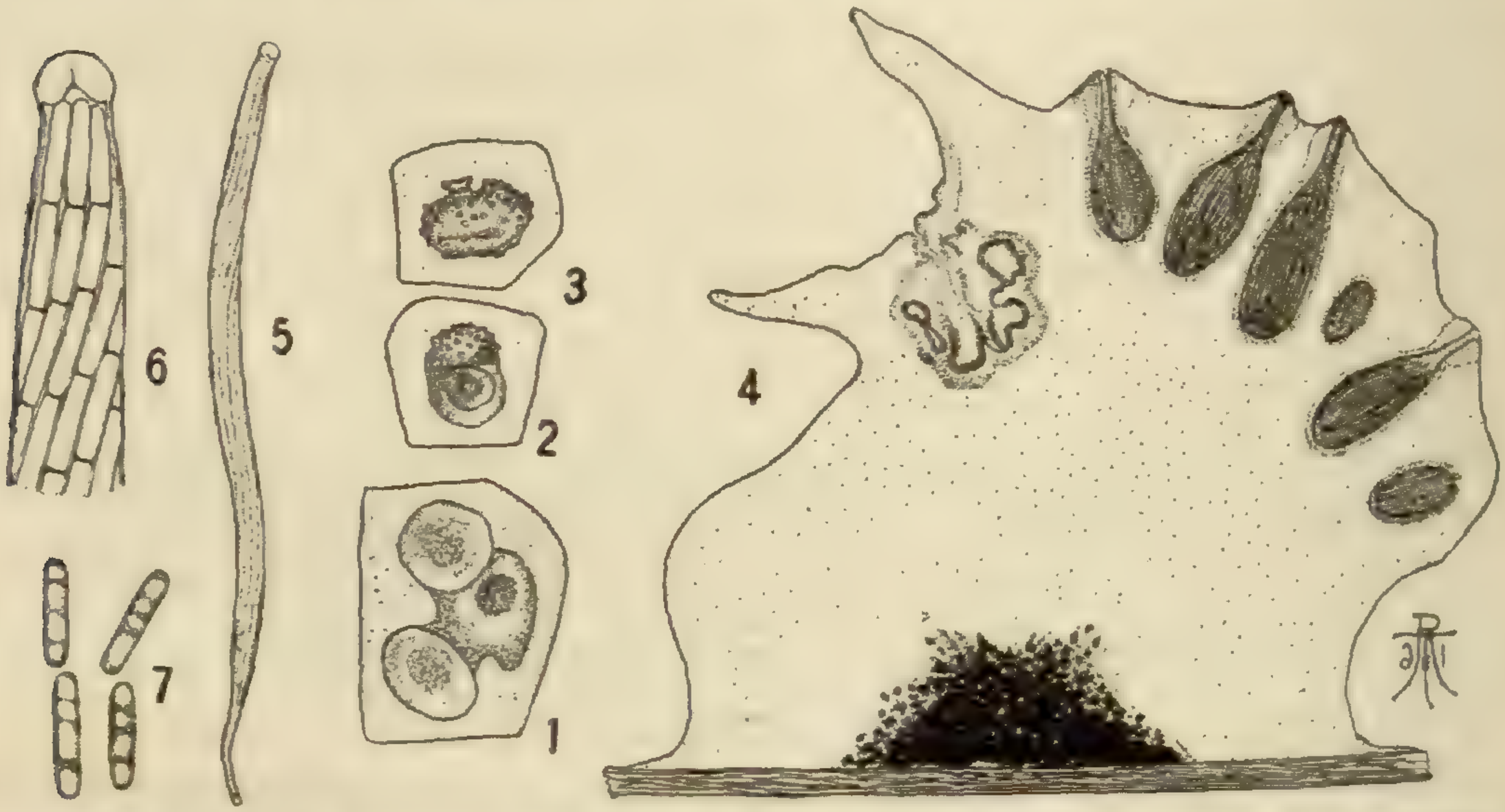
Having assembled a considerable number of *Aschersoniae* from various sources, it was first my intention to attempt a revision of the genus, but an examination of the literature and such material as is available has made it evident that this is hardly possible at

the moment, the great variability of the individual species as regards habit, size, and color, the usually insignificant differences in their spores as well as the absence of any information as to the nature of their true hosts, except in a very few cases, combining to make their systematic study a matter of great difficulty. It has seemed desirable, therefore, in the present connection to attempt no more than a brief preliminary note on the ascosporic stage of *A. turbinata*, a species which, although it is extremely variable in habit, size, and color, is in its typical form quite unmistakable.

Although the perithecial stromata of *Aschersonia turbinata* are less highly specialized than they are in some of the species, the perithecia are usually aggregated in more or less distinct pustules which, more frequently in this than in others, seem to arise after the pycnidial form has practically ceased its activities. Often, however, the whole stroma is perithecigerous, and no pycnidia precede or accompany them. In some cases these perithecial groups are very small, as in fig. 1, where less than a dozen have been produced from an old stroma bearing two well developed pycnidial cups. In fig. 2 a smaller but similar cup is associated with a much more definitely developed perithecial pustule, and fig. 4 shows in section a similar condition. In fig. 3 almost all of the original stroma is perithecigerous, a small pycnidial cup being present at the side, while the perithecia are more scattered. The section of such a specimen (fig. 4) shows a continuous homogeneous stroma, composed in all parts of absolutely identical, closely and intricately interwoven, thick-walled, undifferentiated hyphae; so that, were it not otherwise evident, there can be no question that the perithecia observed are those of the *Aschersonia*, and not of some other fungus parasitic on its stroma. It may here be mentioned, however, that several such parasites have been observed, although their characteristics are quite different.

The perithecial cavities, as shown in fig. 4 at right, are almost completely imbedded in this stroma. They are bottle-shaped, with a relatively narrow and well defined neck, about 440×150 μ, and are surrounded by a more dense, thin perithecial wall, the substance of which is like the similar but broader layer which sur-

rounds the neck and forms the bulk of a definite though not very prominent papilla which marks the position of the perithecium externally, and is perforated by the ostiole. The asci (fig. 5), which arise from a slight cushion at the base of the perithecial cavity, are about 210×7–8 μ, rather slender at maturity, tapering slightly to the peculiarly differentiated apex, which is modified (fig. 6) in a fashion exactly resembling that seen in the asci of *Cordyceps* and its allies. As the asci mature, the stalk becomes more elongate and slender than is represented in fig. 5, and the



FIGS. 1–7.—*Aschersonia turbinata* Berk.: figs. 1–3, three stromata bearing pycnidial cups and perithecial pustules, ×3.5; fig. 4, section of a stroma similar to that shown in fig. 2; a pycnidial cup at the left, the perithecial pustule at the right (the remains of the coccus host in the middle next the substratum (Zeiss A+2); fig. 5, ascus not fully mature (Zeiss D+4); fig. 6, tip of a nearly mature ascus showing segmented spores, and fig. 7, separated spore-segments; both Leitz water im.+12; figs. 4–7 are reduced to one-half.

eight filamentous spores, which are at first cylindrical and continuous, are later divided by septa as in *Cordyceps*. The segments thus formed eventually separating from one another, the ascus becomes filled with countless spores, rodlike in form, about 10–12 ×2–2.5 μ, with rounded ends (fig. 7). The spores and their segments are conspicuously vacuolate, so that they present a banded appearance which gives them a distinct individuality.

The characters briefly enumerated above apply in general to the perfect conditions of the remaining species in which they have

been observed, the specific variations, in so far as I have examined them, consisting in minor differences relating to the distribution, form, and size of the perithecia, the size of the spore-segments, etc., but since, for the reasons above stated, it seems almost impossible at the moment to determine them accurately, any further account of them must be deferred.

HARVARD UNIVERSITY

MORPHOLOGICAL INSTABILITY, ESPECIALLY IN PINUS RADIATA

FRANCIS E. LLOYD

(WITH TWO FIGURES AND PLATE XIV)

The various behaviors of the vegetative and reproductive shoots in the Coniferae have for many years been the objects of extended observation and experiment, and these have been the basis of a massive literature. From this we may derive no mean conception of the amount of morphological instability which characterizes that genus which, in some regards at least, is the most highly specialized of all, namely *Pinus*. Of the conditions which discover such instability, injury has been the most efficient, the resulting unusual developments being said to be due to disturbed nutrition, especially over-nutrition. Precisely what is meant by this is not and cannot at present be stated, so that any light which experiment or the diversity of behavior in nature may afford us should be welcomed, especially if it may lead to a more specific indication of the most potent of the causes which must always be at play.

Evidence of such specific value is given us by the Monterey pine (*Pinus radiata*), a species as definitely restricted to a small area as the famous Monterey cypress (*Cupressus macrocarpa*), a geographical neighbor. The center of this area is, as nearly as may be, at Carmel-by-the-Sea, where is stationed a laboratory of the Carnegie Institution of Washington. The detailed descriptions of California trees in JEPSON'S *Silva of California* render minutiae unnecessary, though it may be noted in passing that among the teratological observations no mention is made in this work of the peculiarities to be noted below.

Carmel is situated in a forest of *Pinus radiata*, not, however, to the advantage of the tree. It is becoming more and more overcome by borers and fungi. It is, in any event, a short-lived tree of small dimensions but very rapid growth, a fact of importance in the present connection. It grows readily under cultivation, and

is very commonly seen in gardens, having either started by chance or by being planted. It is even used as a hedge plant, though of indifferent value for the purpose. But this circumstance leads often to the trimming of young trees, and so enables us to judge of the relative effect of traumatic stimulus as compared with another, namely, the amount of soil water, in producing unusual responses.

It must be prefaced that the amount of soil water in the normal habitat of this pine runs down very markedly during the long growing season, in spite of the moisture-laden atmosphere. Exact measurements are lacking, but the fact is sufficiently evident from the behavior of the vegetation in general, which becomes during the summer months of a distinctly xerophytic character. To this condition is due the gradual reduction in length of the fascicled leaves toward the apex of the season's growth, giving to the foliage of the leaders a cone-on-cone profile. PHILLIPS[1] has observed the same fact in *Pinus cembroides* Zucc., the Mexican piñon, in the mountains of southern Arizona, in a habitat which may, as regards soil moisture at any rate, be compared pretty closely with Carmel.

When grown in gardens, however, it generally happens that a greater abundance of water is provided, toward which a marked response is shown, both in amount of growth and in abnormal behavior. This is most obvious in an open spot used as an experimental garden within the grounds of the Carnegie Laboratory. Here the soil is kept abundantly supplied with water from springs, and here grows a cluster of young trees with heights ranging up to 30 feet or over. Aside from the generally well developed character of these, they all have fascicles which in the majority of cases proliferate. So numerous are the resulting short shoots that the branches become densely clothed, enough so as to quite hide the parent shoot itself from view when looked at from above.

Interest attaches to the phenomenon less because of the morphological fact, since it has long been known that pine fascicles do sometimes proliferate,[2] than because of their abundance and the

[1] Plant World 14:66. 1911.

[2] For the literature on this see THOMSON, R. B., The spur shoots of the pines (to appear in the next issue of this journal), the manuscript of which the author has kindly allowed me to see.

regularity and constancy of their production. They appear, not, as might be expected, when the fascicles are young, but in those three or four years old especially. Unlike the lateral shoots of the whorls of branches, they are always negatively geotropic. This feature is brought out sharply in pl. XIV, *B*, which shows a length of a three-year old branch between whorls. The fact that fascicles as old as three or four years can renew their youth is worth notice. Those of *Pinus Taeda* in Alabama have been found to proliferate after two years, having been stimulated to grow from injury by cattle. This was in the cases of a couple of small trees which were six or eight years old. So far as I have been able to observe, in no instances do the abnormally developed spur shoots become permanent branches in *Pinus radiata*, although that there is evidently nothing in their nature to prevent further development appears from the fact that such is the case in *Pinus Taeda* (fig. 2, *B*). I have thought that the rapid rate of growth of the parent branch and the smallness of the spur shoots rendered successful histological articulation difficult, discrepancies which would be reduced if the parent branch is small to begin with, and of not rapid growth, as is true of *P. Taeda*.

Another example, and a still more striking one, I found at Carmel in the yard of Mr. SLEVIN, who kindly made a photograph for me. Except below, it was entirely without whorls, though a few extra-verticillate but ill-developed branches had grown. This abortion of whorls is quite common in this tree, but has been seen in other Coniferae (PHILLIPS, *loc. cit.*). The tree was growing quite near a cesspool. As the photograph shows, the whole of the chief stem (save for a small stretch) was densely clothed with foliage, due to the proliferation of nearly every fascicle, so that a fox-tail effect was produced. In the lower part of the stem, at the level of the bottom of the photograph, the spur shoots were dying and dropping off. Above they were growing, and the longest had attained a length of several centimeters. It was evident, however, that they were not able to become permanent in character, and there was no evidence that any of the branches had originated from fascicles.

I found no other such examples. Occasionally in small trees

which had been trimmed I saw a tendency for the fascicles to proliferate, but it was quite evident that pruning is by no means as efficient a factor as water supply. At the same time, we are bound to note that precisely where such a supply is abundant, and, in a remarkable case shown in fig. 1, where nitrogen in some form must have been quite plentiful, the development of verticillate branches was arrested. Other cases of absence of whorls were noted at Carmel, but only in gardens, though we know them to occur in nature

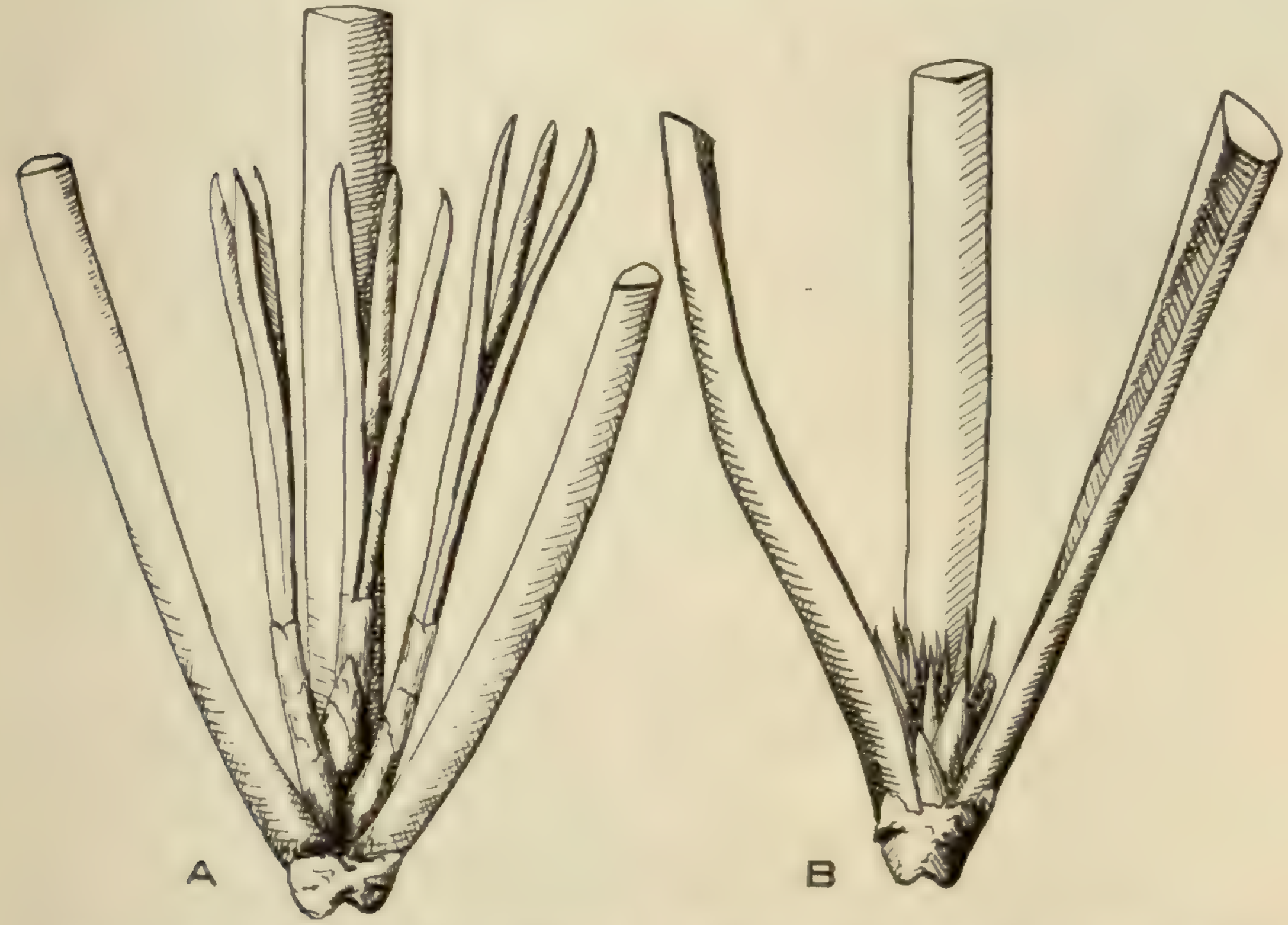


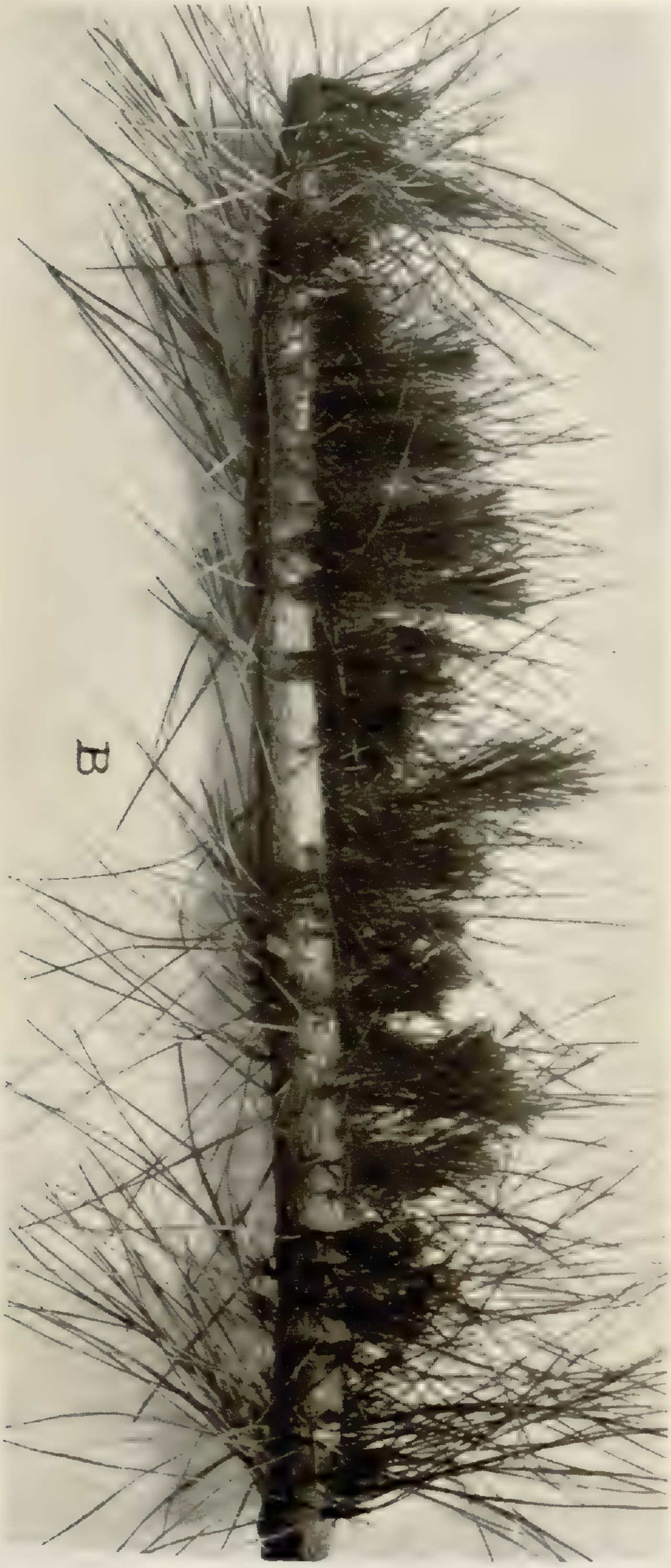
FIG. 1.—Proliferated spur shoots of *Pinus radiata: A*, the shoot so formed produced fascicles at once from the axils of the fascicled leaves of the spur shoot; *B*, hypertrophied scale leaves produced on the proliferating axis.

in other species. It is evident, however, that it is not due to less favorable soil conditions even here, since PHILLIPS (*loc. cit.*) notes that arrest of whorl development occurred in *Abies* on moist, rich sites in Arizona. One may conceive, furthermore, that a very rapid and energetic development of a chief shoot, especially in one in which the lateral shoots are not even laid down during the earlier part of period of growth, might be held responsible for failure to produce the usually formed lateral shoots.

Analogy in support of this view is not wanting, in general support of which may be cited also the apparently rather ready production of proliferations from spur shoots, with or without injury, in pine seedlings found by THOMSON. These seedlings grew in nurseries, probably under unusually favorable conditions for this

FIG. 2.—Proliferated spur shoots of *Pinus Taeda:* *A*, development of the axis below the whorl of fascicled leaves; *B*, a permanent branch formed by proliferation.

reason, especially as regards water supply. The likelihood that the spur shoots of mature trees do not proliferate, or if so more rarely than those of young trees or seedlings, is little lessened by my observations of *Pinus radiata*, since the trees were all young, or at any rate were not mature.



LLOYD on PINUS RADIATA

The character of the shoot produced by proliferation of the fascicular bud is worth further notice. As seen by the text figures, the leaves succeeding immediately on the fascicled leaves may be either true scale leaves (fig. 2, *A* and *B*), or the same hypertrophied (fig. 1, *B*), and hence of juvenile character; or again, new fascicles may be produced at once in the axils of the three leaves of the parent fascicle (fig. 1, *A*), thus showing that fascicled leaves may subtend axillary buds. Far more rare than any of the preceding is the elongation of the axis of the spur shoot below the fascicled leaves instead of that above, as I found to occur in *P. Taeda* after injury. In such cases the leaves of the fasciculate whorl (fig. 2, *A*) do not attain their normal shape and dimensions, but are wider at the base and taper somewhat toward the apex, thus approaching hypertrophied scale leaves in form. Here, therefore, we have arrested fasciculate leaves and over-developed scale leaves approaching a common type, which probably simulates the form of the scattered leaves of the progenitors of the pines.

From the point of view of comparative morphology, it seems logical and in accordance with the facts to argue with THOMSON that the type of fasciculation seen in *Pinus* is a highly specialized condition, derived from a prototype in which the spur shoots are not limited in growth. As THOMSON, however, has taken up this question in the paper referred to, I leave it here. The degree of physiological plasticity displayed by various species of the genus, and especially the amount shown by particular ones, notably *Pinus radiata*, argues, in my own mind, for a comparatively recent origin of the kind of spur shoot characterizing it. The evidence above cited appears to favor the view that abundance of water is of prime importance in disturbing the ordinary equilibrium, and thus stimulating the proliferation of spur shoots.

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EXPLANATION OF PLATE XIV

A, small tree of *Pinus radiata* from which are absent the normal whorls of branches; the dense fox-tail effect is due to very numerous proliferated spur shoots; *B*, piece of a branch of another tree of the same species, showing the numerous proliferating spur shoots.

LIFE HISTORY OF PORELLA PLATYPHYLLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 184

FLORENCE L. MANNING

(WITH PLATES XV AND XVI)

There was no definite knowledge of the morphology of *Porella* until CAMPBELL (3) published an account of the life history of *P. Bolanderi* in 1904. Under the name *Madotheca*, LEITGEB (7) published a few figures of the apical situation. ENGLER and PRANTL (5) barely mention the group to which it belongs, and in their classification it receives the name *Bellincinia* (under the group Bellincinioideae). From 1904 to 1908, the literature is bare of any mention of *Porella*, that is, so far as its life history is concerned. In 1908, ANDREWS (1) accidentally discovered an abnormal situation in the archegonium, finding one with two axial rows, each containing the same number of neck canal cells, and each with a ventral canal cell and an egg. He reported the fact without drawing any conclusion as to its probable meaning. All investigators who have worked with *Porella* agree in regarding it as of high rank among the acrogynous Jungermanniales.

MATERIAL AND METHODS.—The material for the present investigation was collected by Dr. W. J. G. LAND, and some of it had been in the laboratory in a dried condition for several years. In order to revive it, it was soaked for 24 hours in water at a temperature of about 31° C. At the end of this period, it was as fresh as though it had never been dried; and the subsequent examination of the imbedded material showed that it had suffered no ill effects. This ability of liverworts to revive after a long period of desiccation has long been known. CAMPBELL (2) experimented with some Californian liverworts and found them able to recover after having been dried for months. No satisfactory explanation of this phenomenon has been given. GOEBEL (6) mentions the various devices of leafy liverworts for holding water for a long period, such as tubers, water sacs, etc., but this does not explain the power of revival after desiccation.

After the material had been revived, the branches bearing sex organs and those bearing sporophytes were killed in 0.25 per cent chromo-acetic acid, imbedded, and cut in sections 6–8 μ thick. The stains used were safranin, gentian-violet, and orange G; safranin and anilin blue; and iron alum-hematoxylin.

APICAL CELL AND VEGETATIVE BODY.—The apical cell is pyramidal, a type found throughout the Jungermanniales. By pyramidal is meant a cell whose cross-section is an isosceles triangle, and that has three cutting faces. Branches may arise from the latest segment of the cutting cell (fig. 1).

The leafy body is dorsiventral and recumbent, with two dorsal leaves and one ventral leaf (amphigastrium). The dorsal leaves have ventral lobes, which give to the ventral surface the appearance of having three rows of leaves.

The sex organs are borne on short lateral branches, those bearing archegonia being shorter than those bearing antheridia. The sporophyte is surrounded by a cluster of broad leaves.

ARCHEGONIUM (figs. 2–9).—The archegonium arises as a papillate cell from the segment of the apical cell or from the apical cell itself. The first division is transverse; the inner cell is the stalk cell, which does not divide until late in the development of the archegonium; the outer cell forms the archegonium. The first division of this outer cell is vertical, followed by two more vertical walls in rapid succession, cutting off a central cell. Transverse division of the central cell results in the cap cell and the cell which produces the axial row. Divisions of the peripheral cells form a jacket about the central cell and its progeny. The axial row comprises 4–6 neck canal cells in addition to the ventral canal cell and egg.

In the material studied, there was found an abnormal archegonium such as ANDREWS (1) reported (fig. 9). Whether such an archegonium has any bearing upon the question of the origin of the archegonium or not remains to be seen. In any event, it would fit well into the series of hypothetical sketches by DAVIS (4), connecting a gametangium ("plurilocular sporangium") of the brown algae with an archegonium of the liverworts. Miss LYON (8) has described cases of archegonia among the pteridophytes with lateral multiplication of the cells of the axial row.

ANTHERIDIUM (figs. 15–27).—The antheridium arises as a papillate cell from a segment of the apical cell, but never from the apical cell itself. The first division is transverse, the inner cell being the stalk cell, and the outer cell producing the spermatogenous cells and the jacket. The next wall may divide the stalk cell transversely, or both stalk cell and outer cell may divide vertically. If the first division of the outer cell is not by a vertical wall, vertical walls appear in the next two divisions. Periclinal walls then differentiate jacket and spermatogenous tissue. The jacket becomes several cells thick by periclinal divisions, and by further divisions the spermatogenous tissue appears as blocklike masses of cells. At maturity, the stalk of the antheridium is long and slender, and uniformly two cells in thickness.

SPOROPHYTE (figs. 10–14).—Only mature stages of the sporophyte were represented in the material. Great variation in the shape of the foot was observed, from the club-shaped foot illustrated by CAMPBELL (3) to a more or less definite anchor-shaped foot. There is no elaterophore, or any grouping of the elaters, but a general distribution of elaters through the capsule.

I am indebted to Professor JOHN M. COULTER and to Dr. W. J. G. LAND for advice and material during the progress of the investigation.

UNIVERSITY OF CHICAGO

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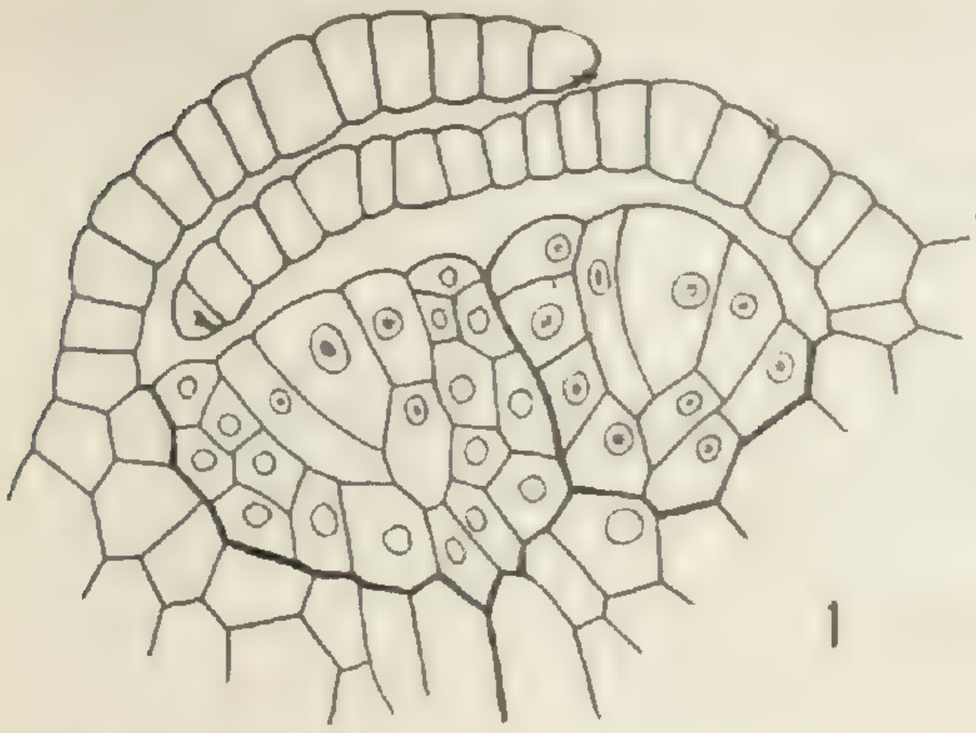

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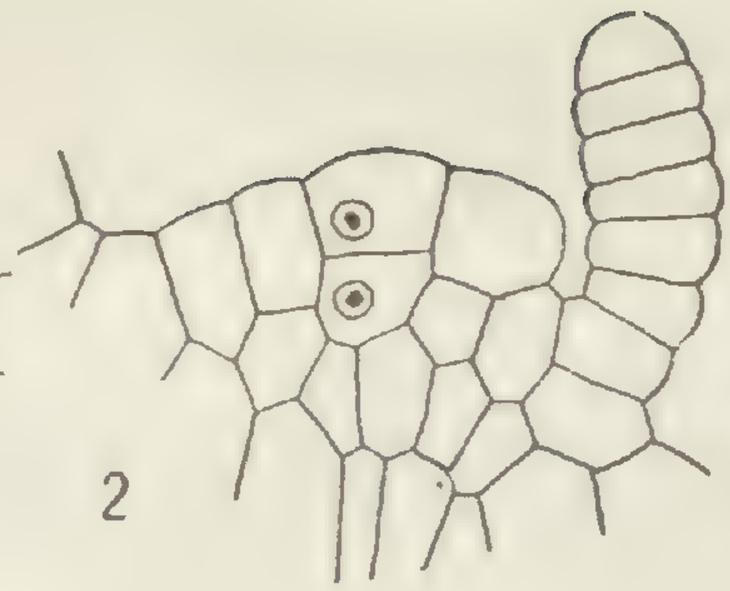

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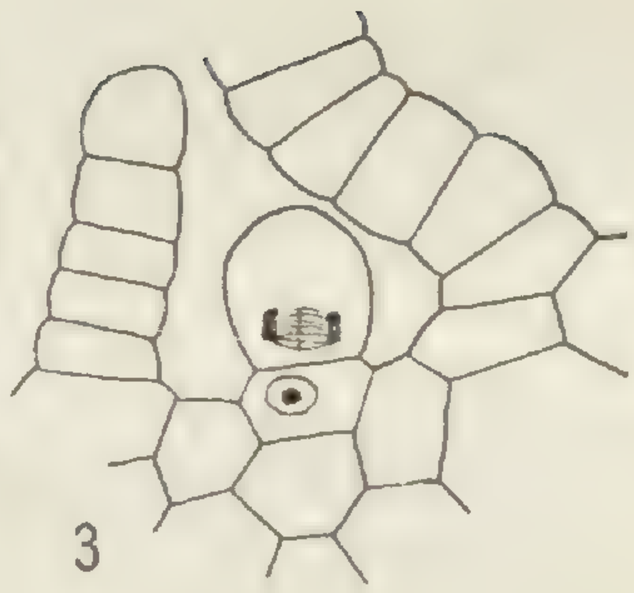

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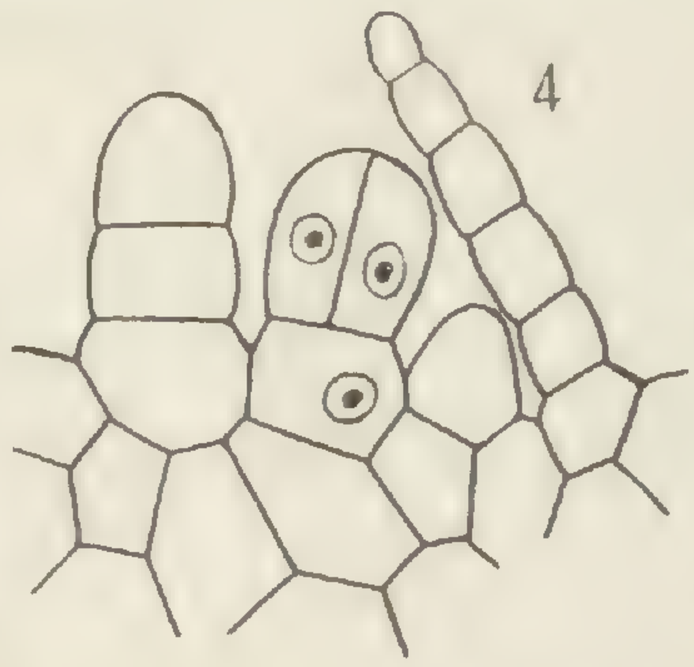

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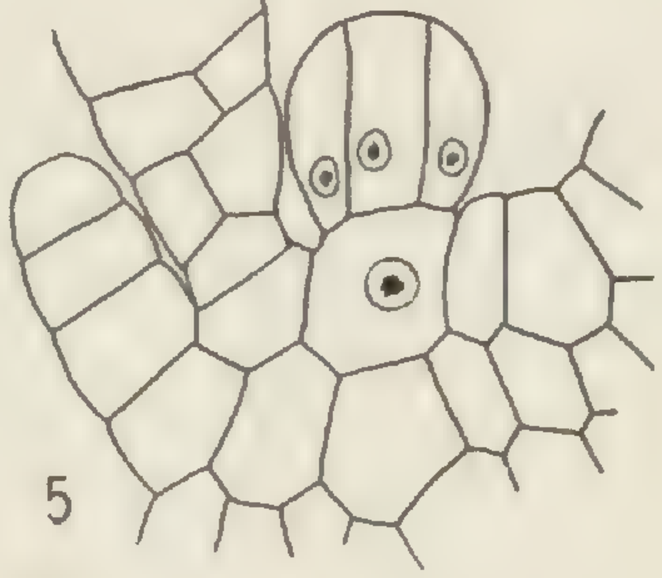

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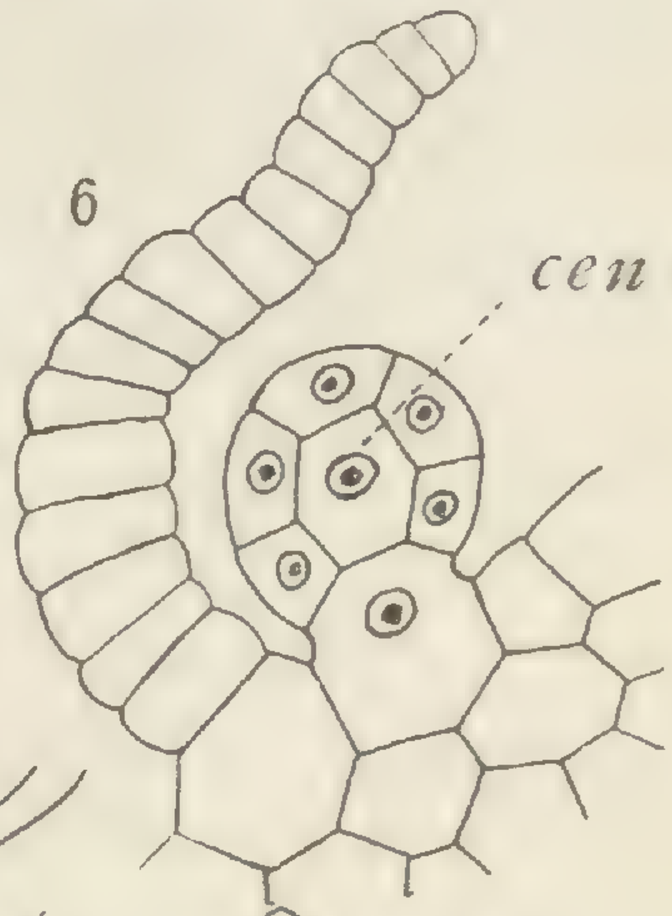

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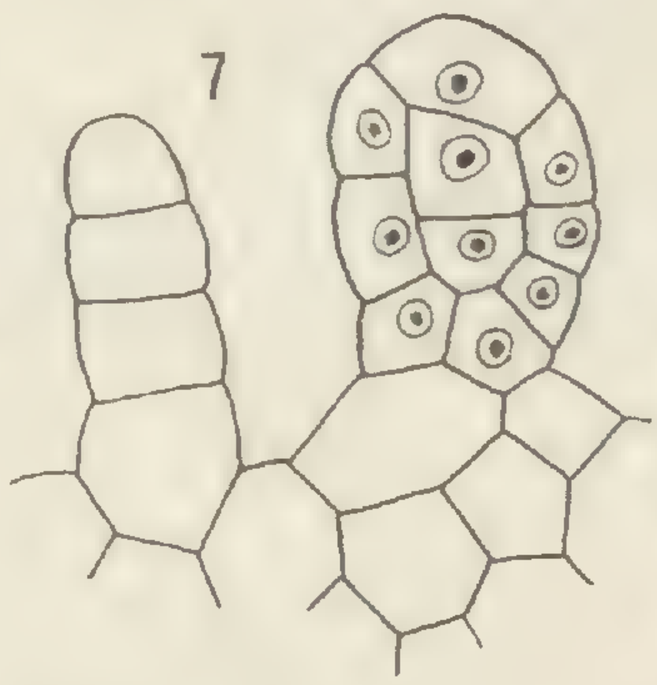

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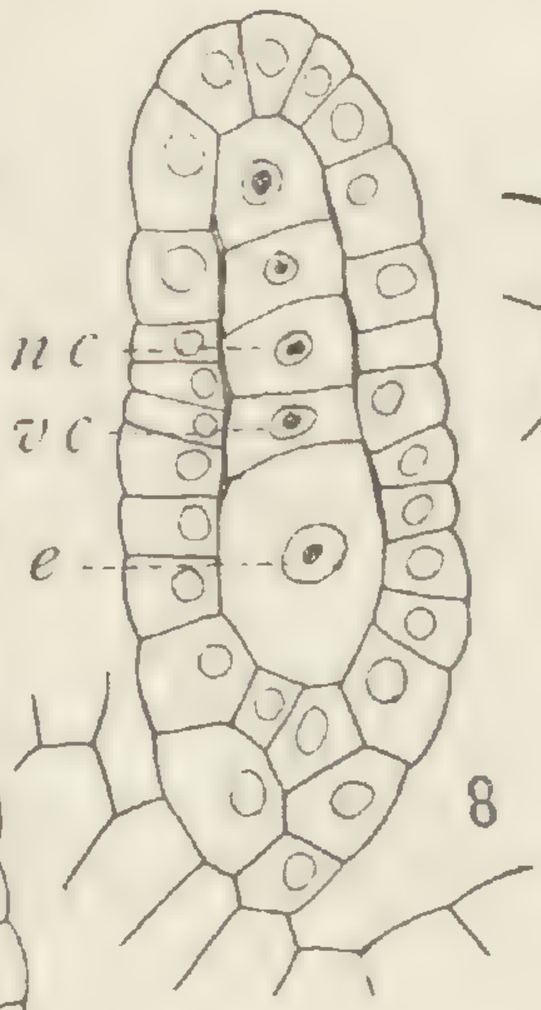

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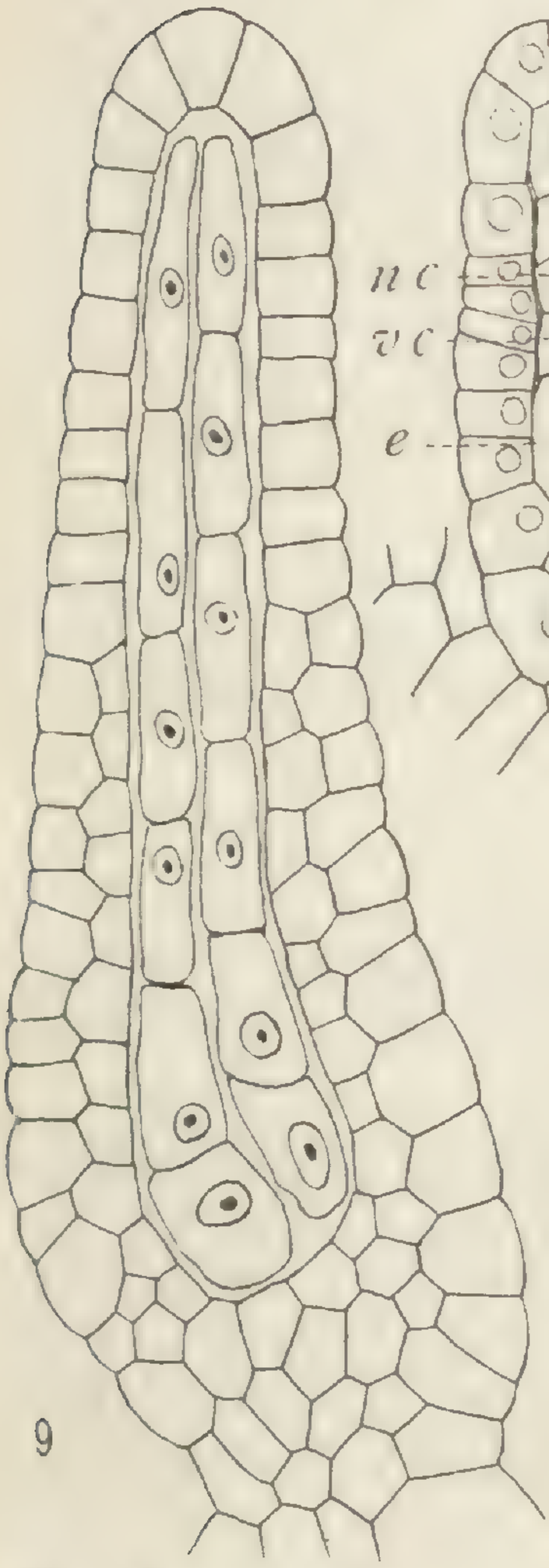

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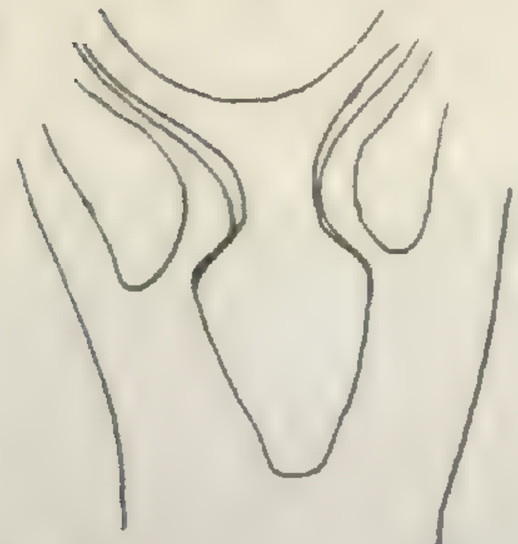

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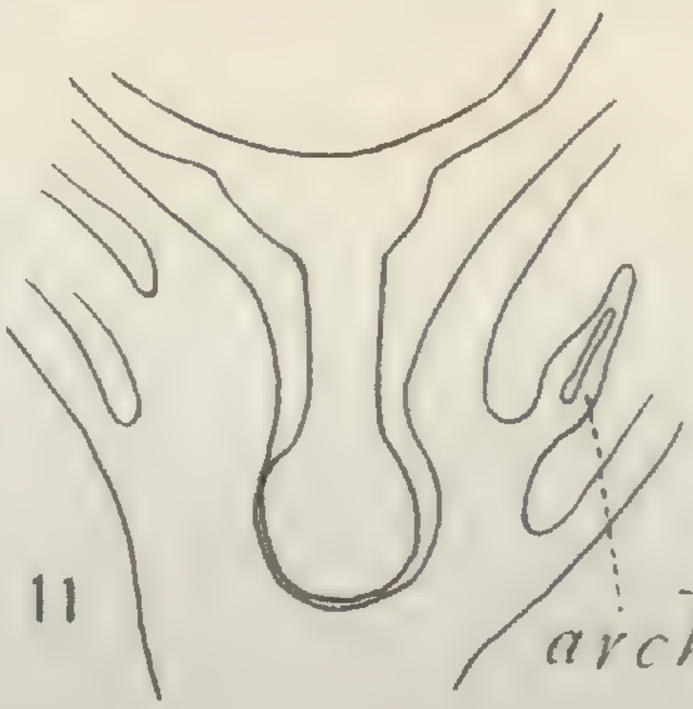

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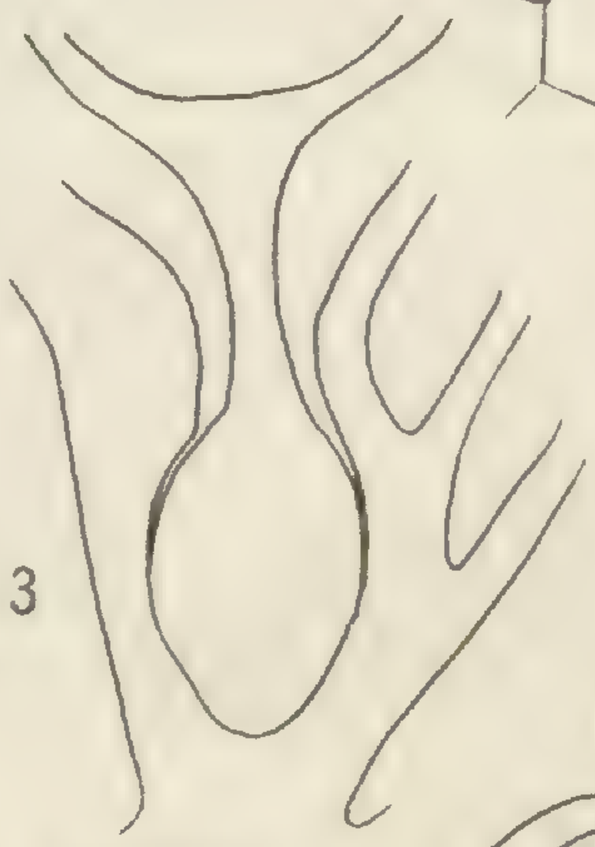

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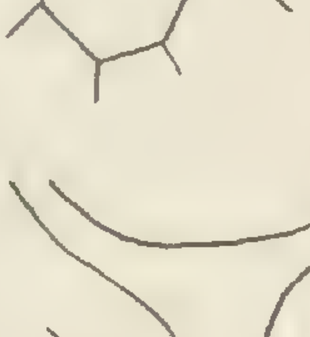

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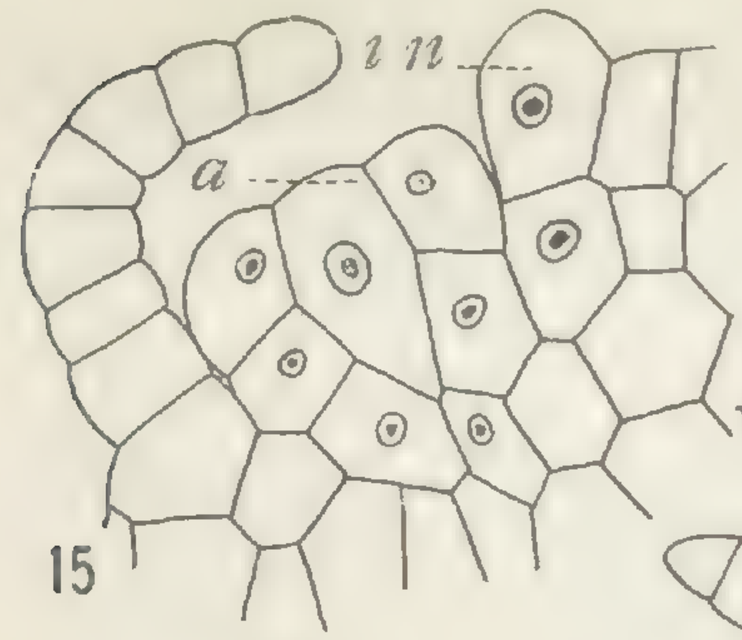
MANNING on PORELLA



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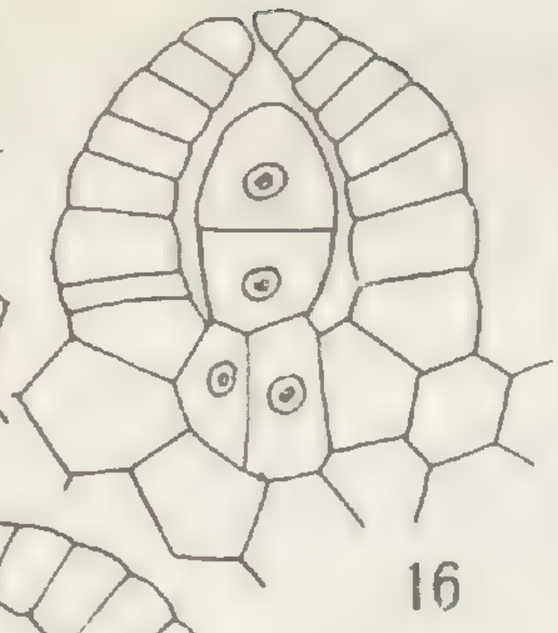
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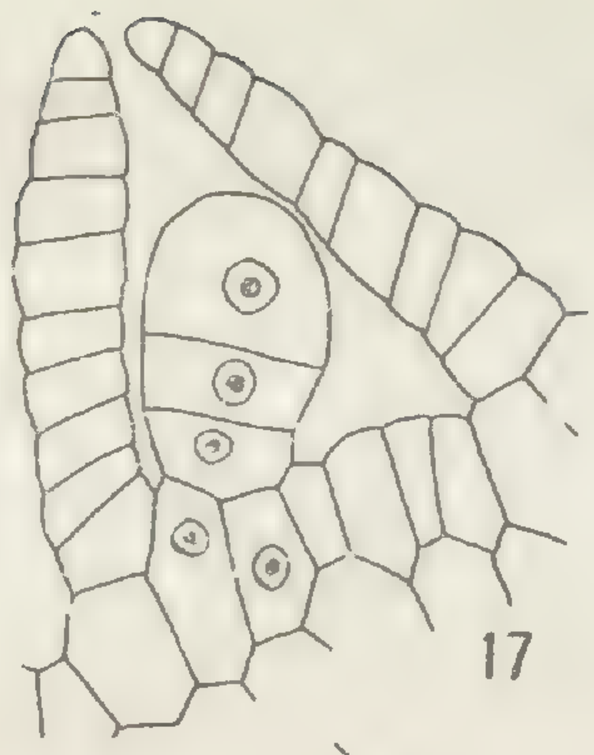
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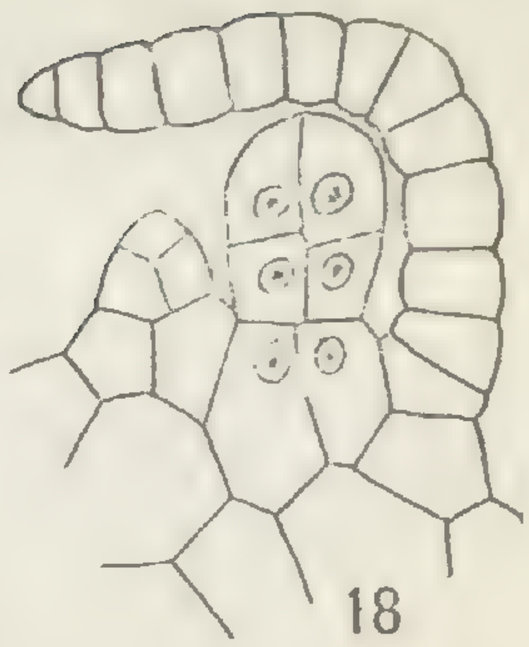
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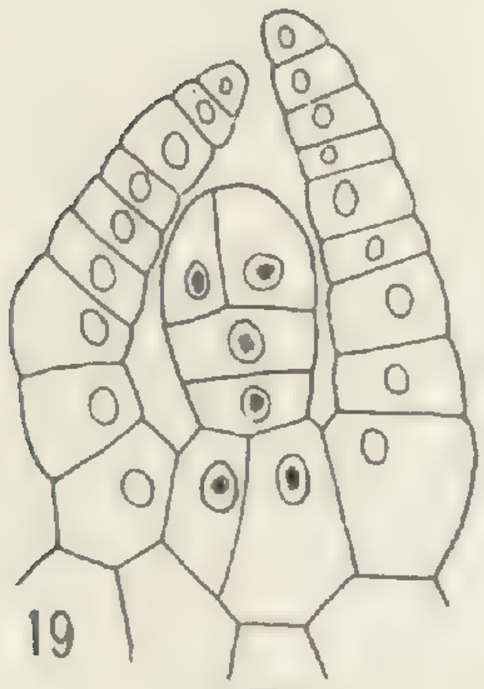
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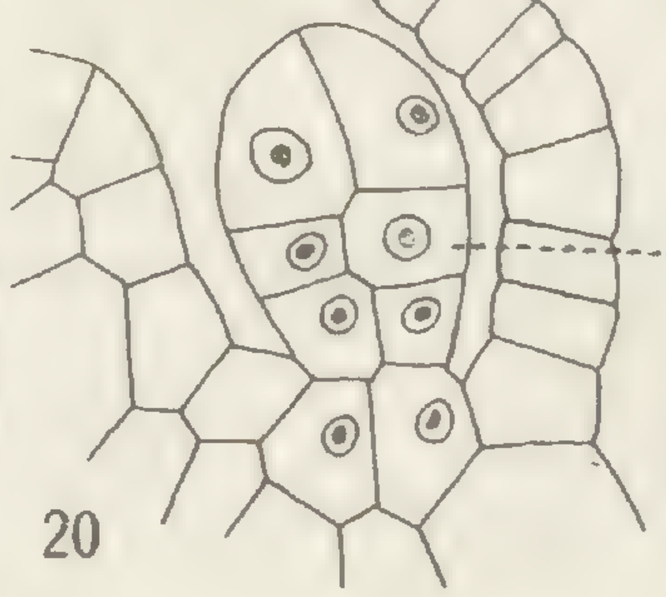
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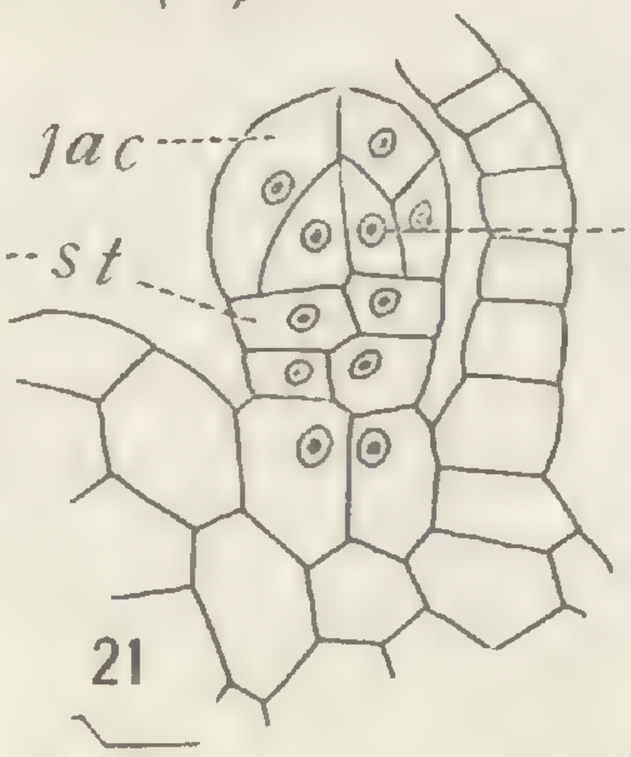

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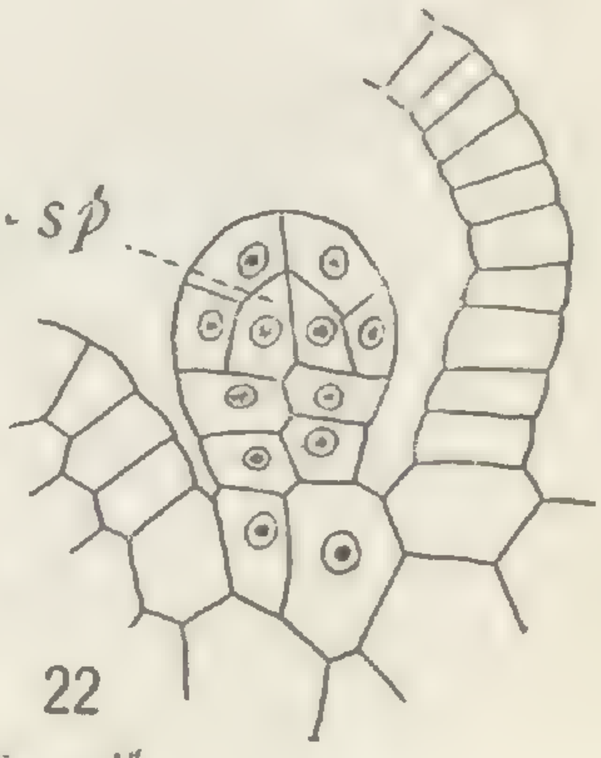

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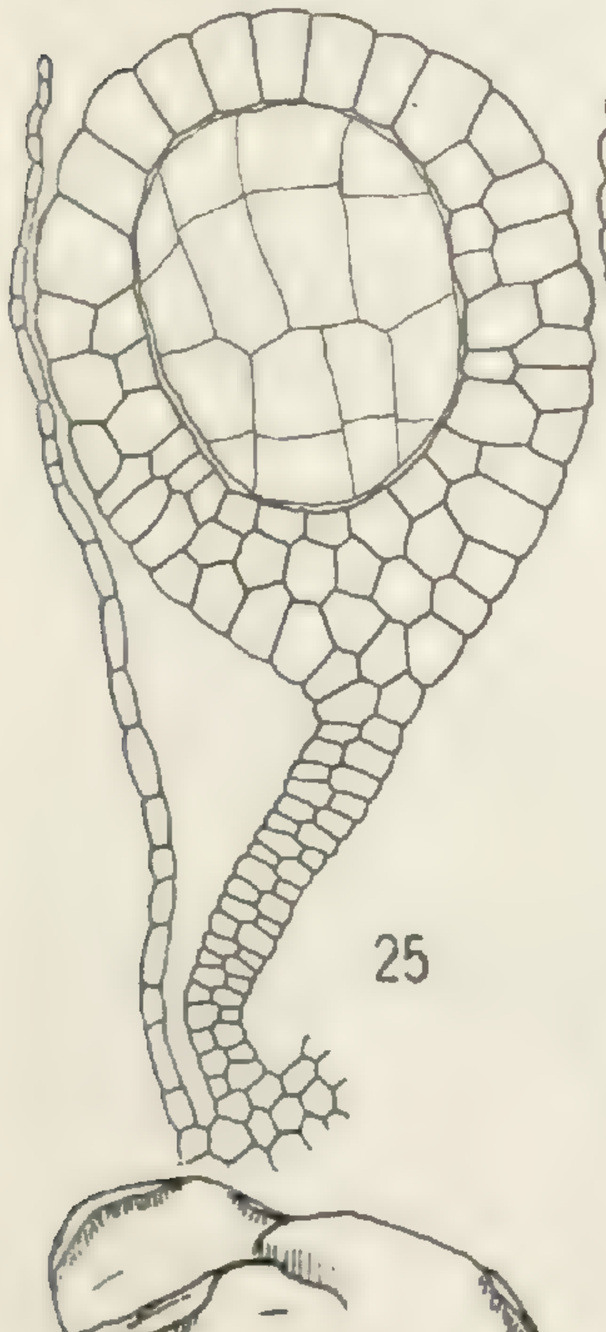
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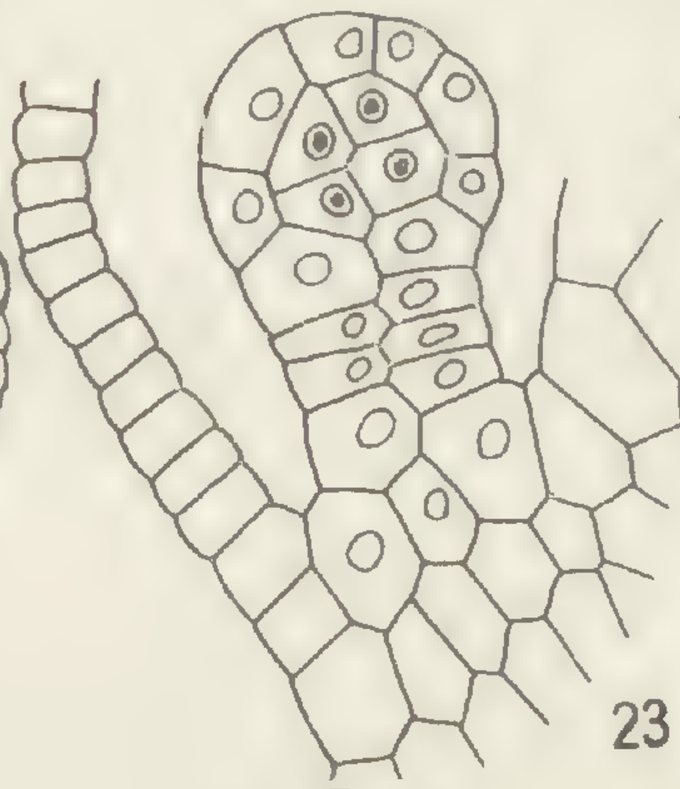
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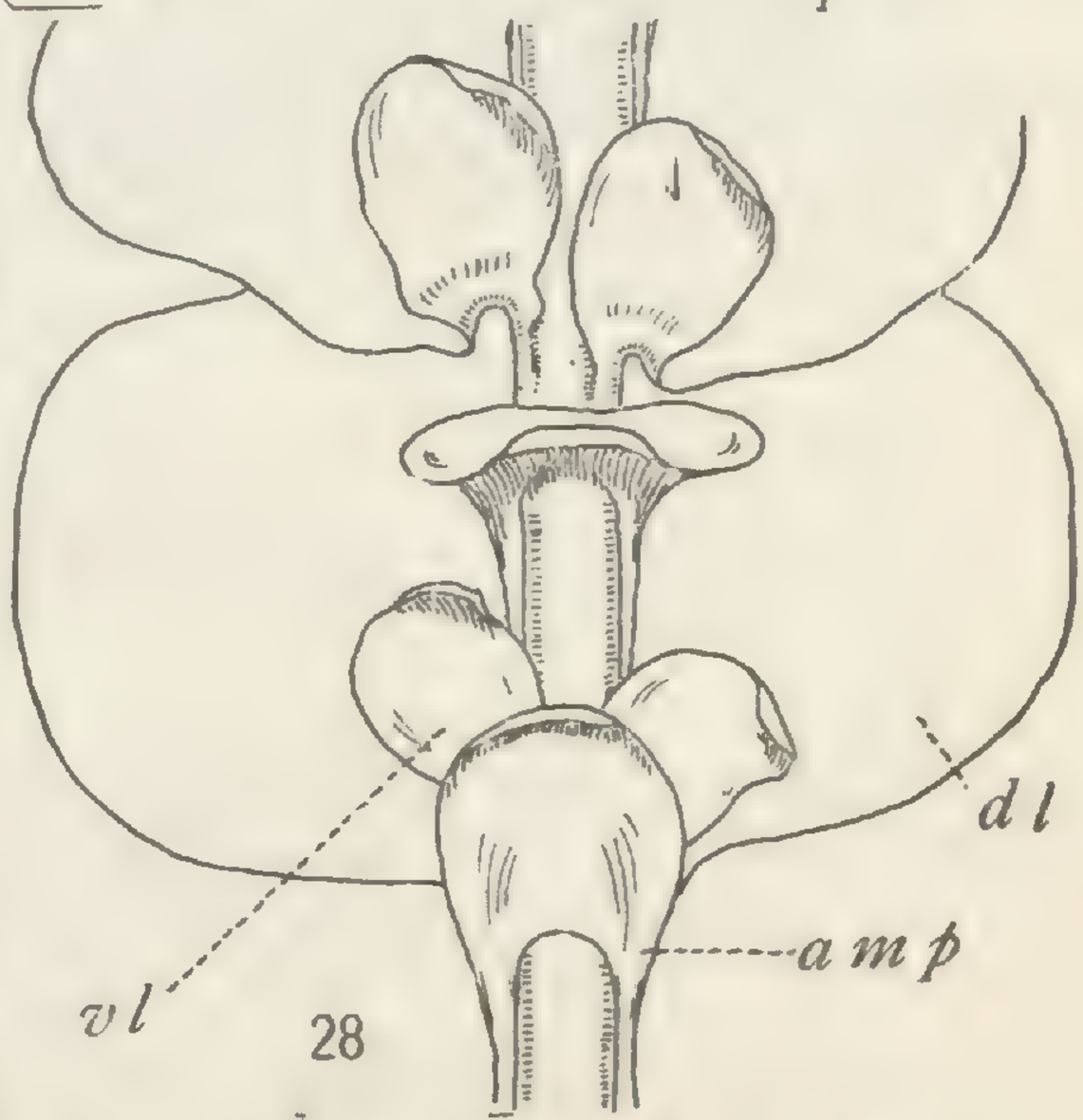

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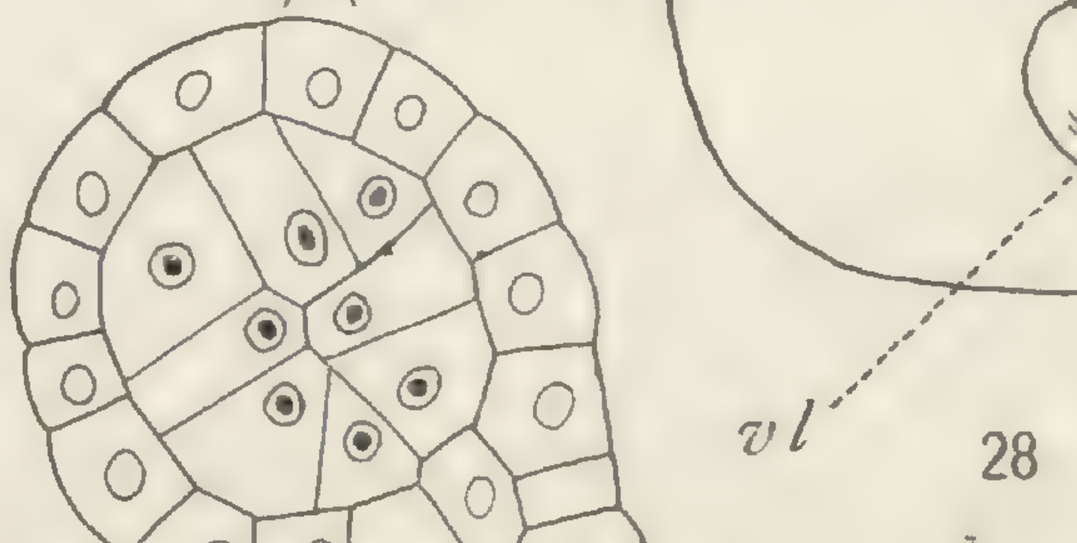
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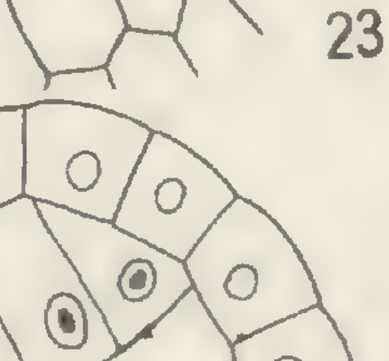
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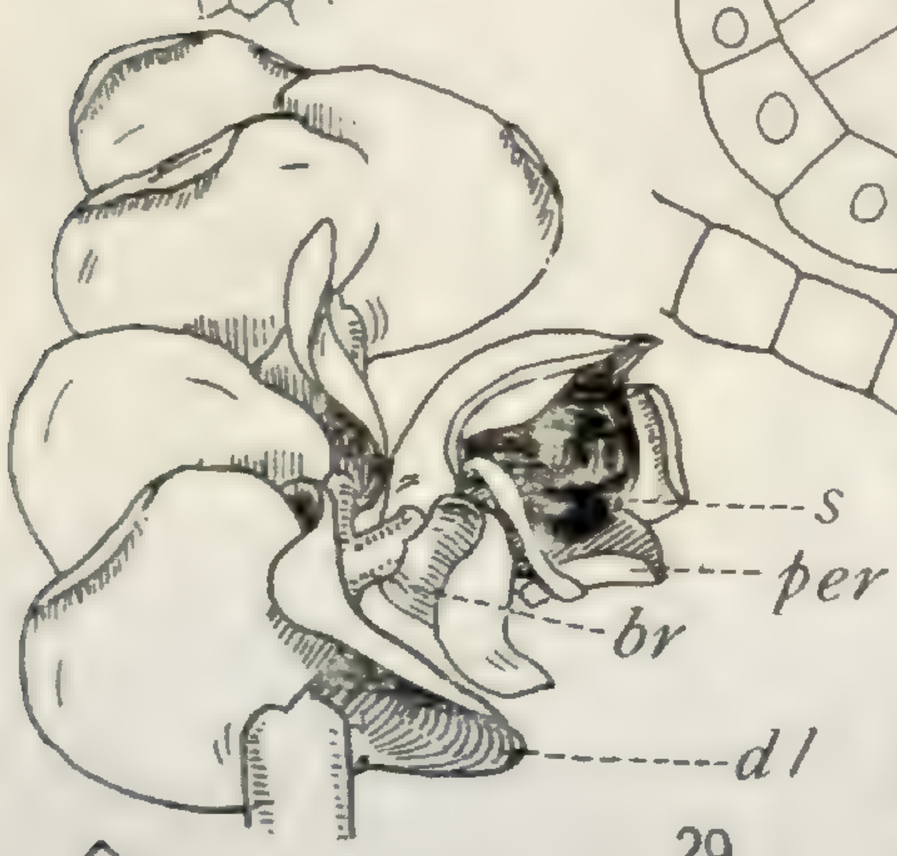

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MANNING on PORELLA

EXPLANATION OF PLATES XV AND XVI

All figures were drawn with a camera lucida, except figs. 25, 28, and 29, and reduced one-half in reproduction. The magnifications appearing in the plates are as follows: figs. 1–8, 14–24, and 27, ×329; figs. 9 and 26, ×115; figs. 10–13, ×22.5.

FIG. 1.—Apical cells, showing branching.

FIGS. 2–9.—Archegonium series.

FIG. 2.—First transverse division of initial.

FIG. 3.—Spindle of first vertical division.

FIG. 4.—First vertical wall in outer cell.

FIG. 5.—Second vertical wall; probably a third one in plane of plate completes the cutting-off of a central cell.

FIG. 6.—Central cell (*cen*) has cut off cap cell; peripheral cells have divided to form jacket.

FIG. 7.—First division of central cell.

FIG. 8.—The axial row, comprising three neck canal cells (*nc*), ventral canal cell (*vc*), and egg (*e*).

FIG. 9.—Mature stage of an abnormal archegonium showing two distinct axial rows.

FIGS. 10–14.—Sporophyte series.

FIGS. 10–13.—Various forms of the foot.

FIG. 14, *a*.—Sporogenous tissue at tetrad stage, before elaters (*el*) are coiled.

FIG. 14, *b*.—Mature spore before shedding.

FIGS. 15–27.—Antheridium series.

FIG. 15.—Antheridial initial (*in*) cut off from the last segment of the apical cell (*a*).

FIG. 16.—First transverse division of the initial cell.

FIG. 17.—The second transverse division; this division may be either transverse or vertical.

FIG. 18.—A case in which the second division was vertical.

FIG. 19.—In this case the second division was transverse, followed by a vertical division in the upper cell; the two lower cells are the stalk cells.

FIG. 20.—Vertical divisions of the stalk cells (*st*).

FIG. 21.—The first periclinal walls cutting off spermatogenous tissue (*sp*).

FIG. 22.—Further division of jacket cells; *sp*, spermatogenous tissue.

FIG. 23.—Division of spermatogenous cells by transverse walls.

FIG. 24.—Further division of spermatogenous tissue and elongation of stalk.

FIG. 25.—Mature antheridium showing blocking of spermatogenous tissue, division of jacket cells by periclinal walls, and the slender stalk consisting of two rows of cells.

FIG. 26.—Portion of a mature antheridium showing simultaneous division of spermatogenous cells.

FIG. 27.—Cross-section of antheridium at stage shown in fig. 22.

FIG. 28.—Ventral view of gametophyte with one of the amphigastria (upper one) turned back to show the lobing of the dorsal leaves; *amp*, amphigastrium; *dl*, dorsal lobe; *vl*, ventral lobe.

FIG. 29.—Dorsal view of gametophyte with one of the dorsal lobes (*dl*) turned back so as to show sporophyte branch (*br*); *per*, perichaetium; *s*, sporophyte.

THE EFFECT OF CLIMATIC CONDITIONS ON THE RATE OF GROWTH OF DATE PALMS

A. E. Vinson

(WITH ONE FIGURE)

The observations on which this study of the effect of climate on the rate of growth of date palms is based were made at the Cooperative Date Orchard, Tempe, Arizona, by F. H. Simmons, at the suggestion and under the supervision of Director R. H. Forbes of the Arizona Agricultural Experiment Station. The length of every leaf on four palms—two Deglet Noors and two Rhars—was carefully measured weekly during 1906 and 1907. By the system adopted the maximum error did not exceed one-quarter inch. In addition to the leaf measurements, daily records were kept of maximum and minimum atmospheric temperatures, and of soil temperatures at one foot, three feet, and five feet below the surface. A record of the level of the ground water was also kept, but this factor probably had no influence on the rate of growth, since the deeper roots were immersed in water at all times throughout the two years.

The data representing the weekly growth of all the separate leaves are too voluminous to use in their entirety. In most cases, after a new leaf has emerged well from the central bud, it makes the greater part of its growth in five or six weeks. After that the weekly growth, as shown by elongation, becomes much less, and finally appears as a negative quantity. This is due to the base of the leaves surrounding the entire stalk, which, as it expands, tends to draw the leaf as a whole lower down on the stalk. After repeated trials to obtain from this mass of data a series of consistent and comparable figures representing weekly growth, it was found that the sum of the elongation of the inner five leaves, that were unfolded sufficiently to be measured, gave the most satisfactory series. These were then calculated for each of the four palms and plotted as a curve, the ordinates of which represent the weekly growths (fig. 1). The maximum and minimum daily temperature,

and the daily soil temperature one foot below the surface at 7:00 A.M. are also plotted.

The temperature factor, as influencing the rate of growth, has other components than those expressed by maximum and minimum alone, because duration of temperature is of the utmost importance. The soil temperature is to a certain extent an index of all these, but in this case is modified by still other factors, such as evaporation. Continuous thermographic records were lacking, so that it became necessary to construct somewhat arbitrary ones. In the dry air and under the clear skies of Arizona the thermographic record is subject to relatively few variations from a normal form. In general the lowest point of the curve falls about sunrise, and the highest at 1:00 or 2:00 P.M. By the use of these points a fairly accurate thermographic record for this region can be drawn from the daily maximum and minimum temperatures. This alone, however, furnishes no usable data for the construction of a curve representing the total daily amount of heat received. If we assume some empirical temperature as that below which no marked growth takes place, and use this as a base line on the thermograph sheet, the areas lying above this line represent, at least relatively, the heat available for growth.

The selection of such a base line is not an easy matter, and at best must be somewhat arbitrary. The data obtained, however, will be relative and consistent on any base line chosen. For the present case 50° F. was selected, because the date palm does not seem to utilize temperatures below that, at least to any marked degree. Growth after the surface soil temperature reached 50° F. was practically nothing. This is not to be construed as meaning that no growth would occur at a uniform temperature of 50° F., but under actual climatic conditions the minimum temperature which would accompany a maximum of 50° F. would effectually inhibit growth. It will be noticed in this connection that during the first week of January 1906 growth was entirely inhibited, while during the winter of 1907, when the daily maximum was always above 50° F. and the minimum seldom below 30° F., growth never entirely ceased.

The curve representing the weekly heat-time areas, when plotted

along with those representing the growth of the palms, coincides in general with them, with one exception. Both years the rate of growth was maintained late into the fall considerably in excess of the amount of heat available. This may have been due to the continuance of the great activity which the plant experienced during the late summer and early fall months; that is, it was in better condition to utilize the available heat at this time than at a corresponding period in the spring.

The curves for the rate of growth show it to be the most active, not at the period of highest maximum, but rather at that of the highest minimum temperatures, which means warm nights. This falls in July, August, and sometimes September; and it is at this time that weakly palms recover their vitality. By far the greater part of the total yearly growth falls in the last half-year. The spring growth is much less luxuriant than the fall growth. The warm nights of July and August are due to the somewhat increased humidity, which prevents the usual rapid radiation after sundown. Humidity in itself is undoubtedly an important factor, but probably has less to do with the rate of growth of date palms than with other plants.

The chief relations of temperature to the rate of growth of date palms according to these measurements are: first, the period of maximum growth coincides with that of highest minimum rather than with that of highest maximum temperature, and this falls during the summer period of highest relative humidity; second, the rate of growth throughout the entire year is, in most cases, in proportion to the heat-time units over 50° F.

The rate of maturation of the fruit is probably influenced by the same factors as the rate of growth of the foliage. The effect of high minimum temperature in promoting the maturing and ripening of the Deglet Noor date has recently been called to the writer's attention by M. BRIQUEZ, the civil governor of Gafsa in Southern Tunis. The oasis of Gafsa, which lies not over 75 miles northeast of the Djerid region, where the finest Deglet Noor dates are produced, produces only second quality fruit. The difference in altitude between the two oases is about 900 feet, Gafsa being 1126 feet and Tozeur in the Djerid 197 feet. The two are separated by

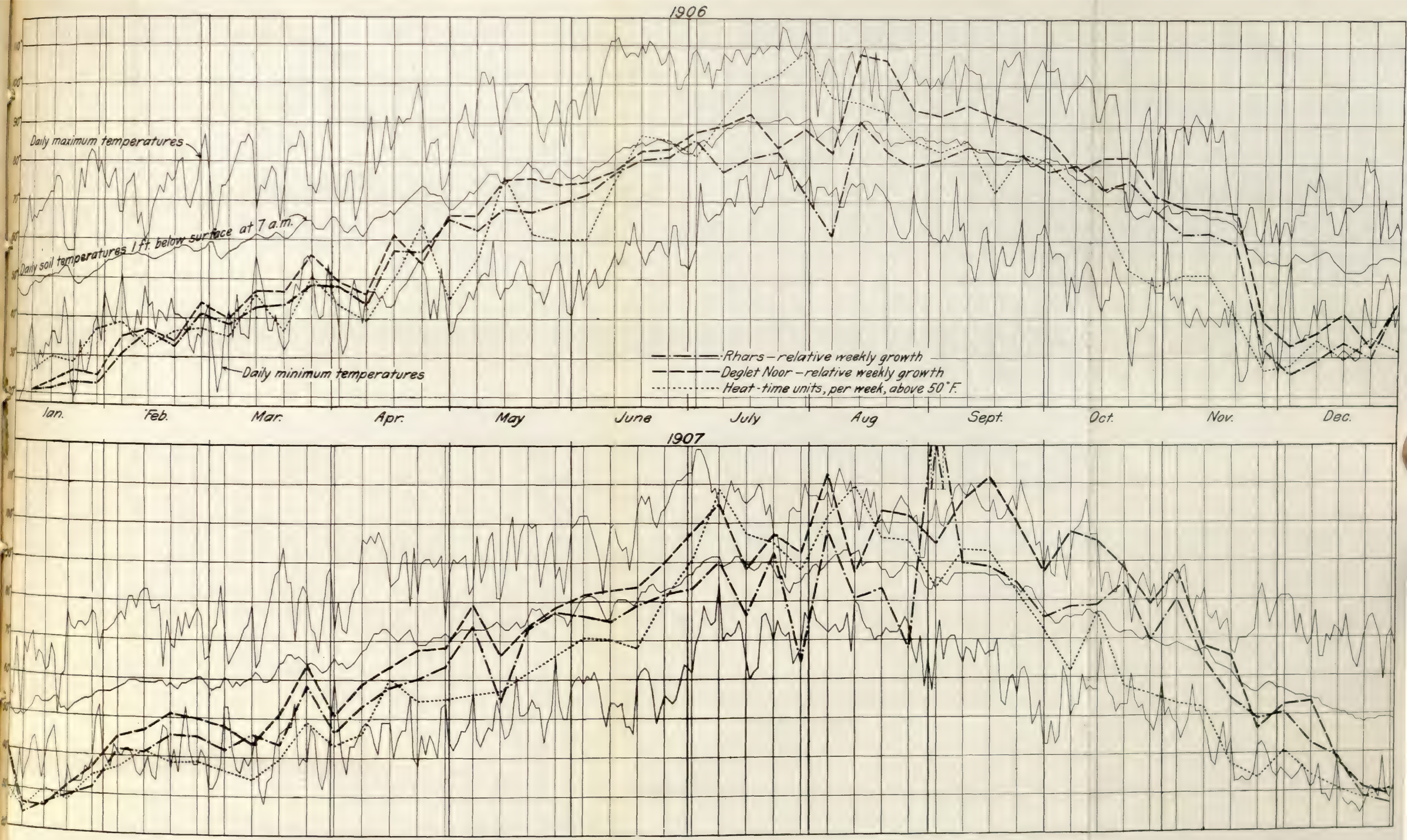

FIG. 1.—Curves showing the relation between temperature conditions and the rate of growth of Deglet Noor and Rhars date palm

several ranges of very low mountains, but the Djerid is exposed to the Sahara on the south. The result is that while the day temperatures at Gafsa are only 6 or 7° F. cooler than at Tozeur, the night temperatures vary by 30 or 35° F. Thus, high minimum temperature seems to be a more important factor in determining the maturation of the fruit than high maximum temperature. This observation is in perfect harmony with the curves for rate of growth obtained at Tempe, Arizona.

ARIZONA AGRICULTURAL EXPERIMENT STATION
TUCSON, ARIZONA

BRIEFER ARTICLES

THE TYPE SPECIES OF DANTHONIA

In a recent paper[1] by NELSON and MACBRIDE the generic name *Pentameris* Beauv. has been taken up for *Danthonia*, and our American species have been transferred with the corresponding new combinations. A quotation from this article explains the authors' reasons for these changes.

As shown by PIPER in his "Flora of Washington" (Contrib. Nat. Herb. 11:122), the name *Danthonia* is not available for the American species that have passed under that name. In choosing among the several later names that have been proposed, he selects *Merathrepta* Raf. in Seringe, Bull. Bot. 1: 221. 1830, apparently because the type species of the genus was *M. spicata*, an American species closely congeneric with ours. But will this fact permit our ignoring *Pentameris* Beauv. Agrost. 92. *t. 18.* 1812, the type species of which is accepted as a *Danthonia*, as that genus has until lately been understood?

I think the authors are justified in taking up *Pentameris* Beauv. in place of *Merathrepta* Raf., but I do not agree with them nor with PIPER in rejecting *Danthonia*, and I take this opportunity of recording my reasons for retaining the latter name. NELSON and MACBRIDE apparently have not investigated on its merits the validity of *Danthonia* for our species, because they say "as shown by PIPER." In the article cited, PIPER merely states: "The type of *Danthonia* DC. is *Festuca decumbens* L. (*Triodia decumbens* R. Br.), and the name cannot therefore be used in the current sense. *Merathrepta* has for its type *M. spicata* (L.) Raf. (*Avena spicata* L.)." With this statement I do not agree, and I will give my reasons. I believe that stability in botanical nomenclature is greatly aided by the adoption of the type method; that is, that for nomenclatorial purposes a genus shall be based upon a type species and a species upon a type specimen. The selection of the type species of a genus fixes the application of the generic name to the group containing the type species. It is easy to determine the type species if the genus is monotypic or if the author has indicated the type. In other cases it

[1] Western plant studies II. BOT. GAZ. **56**:469. 1913.

may be easy to select the type from several species by some statement of the author or because one species was figured. There are cases in which a careful weighing of evidence is necessary to determine the species which the author had chiefly in mind when establishing the genus, that is, the type species. On account of the bearing it may have on the selection of type species, I give here, somewhat fully, the evidence which leads me to select *Avena spicata* L. instead of *Festuca decumbens* L. as the type species of *Danthonia* DC.

DeCandolle[2] establishes the genus *Danthonia* in a local flora and hence describes only the two species growing in France. These are (1) *D. decumbens*, based upon *Festuca decumbens*, and (2) *D. provincialis*, a change of name for *Avena calycina* Vill. In a note at the end of the generic description, and preceding the descriptions of the species, is the following: "On doit, outre les espèces décrites plus bas, rapporter à ce genre, 1°. avena spicata L. ou avena glumosa Michaux; 2°. avena calicina Lam. non Vill." It is evident from this note that the author included these two species in his idea of the genus *Danthonia*. The only reason for selecting *D. decumbens* for the type of the genus is that it is the first of the two species described. There are more and better reasons for selecting *Avena spicata* as the type. It is the first species mentioned and it represents better than *D. decumbens* the generic idea. In regard to the last point, it is to be noted that the generic description states that the lemma ("valve externe") is provided with an awn, sometimes long and twisted, sometimes rudimentary (à demi-avortée). DeCandolle differentiates *Danthonia* from *Melica* by the presence of the awn, and from *Avena* by the position of the awn, and by some other characters. It is evident that the author considered the awn to be one of the important distinguishing characters of his new genus. Three of the four species mentioned by DeCandolle are congeneric and possess a well marked awn. In the other species, *D. decumbens*, the awn is rudimentary ("les valves externes des balles ont au sommet une echancrure d'où part un rudiment d'arête") and hence this species, inasmuch as it departs from the general idea of the genus, should be excluded from consideration in the selection of the type. For the reasons given I favor selecting *Danthonia spicata* (L.) DC. as the type species of *Danthonia*, thus retaining this generic name for our American species. *Festuca decumbens* L. is generally recognized as generically distinct and has been made the type of *Sieglingia*. Some botanists are inclined to select the type from among those species that from the standpoint of the author of the work

[2] In Lam. and DC. Fl. Franç. 3:52. 1805.

are natives. Other things being equal, it is well to do this. But even by this method, the type would be *D. provincialis* DC., as this corresponds better to the generic description than does *D. decumbens*. This selection of the type also retains the name in the traditional sense.—A. S. HITCHCOCK, *U. S. Dept. Agriculture.*

A METHOD OF HANDLING MATERIAL TO BE IMBEDDED IN PARAFFINE

(WITH ONE FIGURE)

The account given by Mr. W. DUDGEON under the above title (BOT. GAZ. **57**:70–72. 1914) has suggested that some might be interested in a similar, but simpler, method which I find useful.

The specimens to be imbedded in paraffine are strained from the killing solution into a small piece (2–4 inches square) of cotton chiffon held in bag form. The corners and sides are then drawn together and tied with a piece of sewing thread (about no. 50), making such a bag as is represented in fig. 1. All surplus chiffon is cut away and the bag is put for washing in a dish into which a rubber tube brings water slowly from a faucet. Any number of bags can be washed in one dish, their contents being labeled by a small slip of paper inclosed in the bag with the specimens. The end of the thread with which the bag is tied is left a few inches long, and by this the tiny bags can be lifted from one solution to another until the specimens are ready to be put into paraffine. The end of the thread left hanging over the top of the bottle does not interfere with replacing the stopper. When the specimens are ready for the paraffine, the bag is cut off just below the thread tie. It spreads open instantly as a flat piece of chiffon, from which the specimens can easily be transferred to the paraffine.

FIG. 1

The chiffon found best is the thin cotton quality usually sold at the veiling counter of department stores at 50 cents a yard; the meshes are 0.25–0.33 mm. in diameter. The material is so thin that the specimens are as good as free in the solution jars, yet any number can be handled with the minimum of labor and time.—ELDA R. WALKER, *University of Nebraska.*

A CORRECTION

In a review of the second edition of JOHANNSEN'S *Elemente der exakten Erblichkeitslehre* in the last number of this journal (BOT. GAZ. **57**:239. 1914), I made a very stupid error for which I wish to apologize to Dr. JOHANNSEN and to the botanical public. From the preface, in which JOHANNSEN disclaims being a "pure Weismannian," and from the sympathetic treatment of SEMON in one of the chapters on the influence of environment, I obtained the idea that he retained a Lamarckian attitude, although disclaiming belief in experimental proof of the inheritance of acquired characters. Oddly enough, other readers of the book gained the same impression, as I learned from various conversations. This phase of the subject being necessarily somewhat ancient biological history I passed it by rather hurriedly. Recently I have read the two chapters concerned carefully, and find that JOHANNSEN is even more of a Weismannian in his essential principles than I had thought. His wish not to be considered as a "pure Weismannian" evidently rests entirely upon his opposition to WEISMANN'S morphological conceptions, as I stated in the review.—E. M. EAST.

A CORRECTION

The new combination *Lobaria oregana* (Tuck.), made in BOT. GAZ. **56**:497. 1913, I find was published in *Flora* **47**:364. 1889, by MÜLLER, and therefore stands **Lobaria oregana** (Tuck.) Müll. Arg.—R. HEBER HOWE, JR.

CURRENT LITERATURE

BOOK REVIEWS

The flora of Western China

A most interesting account of the experiences of a naturalist in Western China during several years of exploration has been given by WILSON.[1] Much of the work deals with the manners and customs of the non-Chinese peoples inhabiting the borderland region that was being explored, but there is also a great deal of botanical material, as the result of four expeditions. An introduction is written by Professor CHARLES S. SARGENT, in which the tree families of Eastern North America and of China are contrasted. The general statement is made that the trees of Eastern North America are larger and more valuable than the related Chinese species, but that Chinese shrubs in general produce more beautiful flowers than those of Eastern North America. In considerable detail 129 families are presented, which occur in Eastern North America and in China. Of these, 92 families are common to the two regions; 12 occur in Eastern North America but not in Eastern Asia; and 25 occur in Eastern Asia but not in Eastern North America. In making a similar contrast with the genera, it is found that out of 692 tree and shrub genera in the two regions, 155 are common to both; while 158 occur in Eastern North America and not in Eastern Asia, and 379 are found in Eastern Asia and not in Eastern North America.

The first volume has chiefly to do with the topography of the region and the character and customs of the peoples inhabiting it. The material of botanical interest occurs chiefly in the second volume, the nature of it being indicated by the following chapter titles: the flora of Western China, a brief account of the richest temperate flora in the world; the principal timber trees; fruits, wild and cultivated; Chinese materia medica; gardens and gardening; favorite flowers cultivated by the Chinese; agriculture; the principal food-stuff crops; the more important plant products; wild and cultivated trees of economic importance; the more important plant products; cultivated shrubs and herbs of economic value; tea and "tea-yielding" plants; the tea industry for the Thibetan markets.

The book is very suggestive of the botanical "travels" of years ago, which have always been the first introduction of botanists to a new region.—J. M. C.

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[1] WILSON, E. H., A naturalist in Western China. 8vo. 2 vols. pp. xxxvii+251 and xi+229. *figs.* 111. New York: Doubleday, Page & Co. 1913.

Flora of Manila

In no place in the tropics has botanical work made such great strides as in the Philippines. This is particularly true of the systematic botany work of the Bureau of Science under the energetic direction of ELMER D. MERRILL. Until recently no effort had been made to collect the numerous taxonomic papers into a flora for any particular region. *The flora of Manila*[2] is not a sufficiently comprehensive title, because the work really covers the flora of the more populated coastal regions of the entire archipelago. About 1000 species, or approximately one-sixth of the total number of species known from the islands, are described. These are distributed among 591 genera and 136 families.

The bringing together under one cover of one-sixth of the known flora of the Philippines will make useful a large number of descriptions of plants that heretofore have been practically inaccessible to anyone except the specialist. The usefulness of the work to the layman is enhanced by definition of terms used in descriptive botany (pp. 9–20), some remarks on classification (pp. 20–21), directions for preparing botanical specimens (pp. 21–23), some remarks on the preparation of the material for the herbarium (pp. 24–25), and a glossary of technical terms (pp. 25–33).

The flora includes practically all the species of vascular cryptogams and flowering plants growing naturally within the Manila district, and most of the cultivated forms both of Philippine and of foreign origin. In it one can find descriptions of nearly all the useful and ornamental plants of the Islands, except the timber trees.

It is to be hoped that the recent reorganization of the scientific staff of the Bureau of Science will not materially interfere with progress in this kind of work. Another five years ought to bring forth a flora of the Philippine Islands.—H. N. WHITFORD.

A plant physiology

A plant physiology by KOLKWITZ[3] offers several features that are novel for a book bearing its general title. Unusual emphasis is given to the lower forms; 60 pages are devoted to the physiology of "phanerogams" and almost 200 to the study of "cryptogams." This change of emphasis has the advantage of bringing into prominence such cosmic cycles as the nitrogen cycle without in any way detracting from an understanding of other physiological processes. The book, however, can hardly be called a plant physiology. It contains many

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[2] MERRILL, E. D., The flora of Manila. pp. 490. Bureau of Science, Manila. 1912.

[3] KOLKWITZ, R., Pflanzenphysiologie, Versuche und Beobachtung en an höheren und niederen Pflanzen einschliesslich Bakteriologie und Hydrobiologie mit Planktonkunde. V. 8vo. pp. 258. *pls. 1–12. figs. 116.* Jena: Gustav Fischer. 1914.

pages of systematic descriptions in connection with the plankton work, including both plant and animal forms; plant morphology receives some attention, and bacteriological and plankton technique are more or less fully described. One is doubtful whether the physiological viewpoint even prevails.

The book is an outgrowth of exercises that have been used in teachers' courses for fourteen years, involving at least 25 repetitions. In spite of this, one is unable to judge whether it is more a laboratory manual or a descriptive text. It seems poorly suited for either.—WILLIAM CROCKER.

Diseases of tropical plants

COOK[4] has published a timely volume which introduces us in a compact way to the diseases of the tropics. The study of plant pathology has been chiefly with the crop plants of the temperate regions, but with the growing interest in tropical plants, there must come a knowledge of the tropical diseases. This vast field has yet to be developed, but the scattered literature that does exist should be brought together, and this COOK has done in a very effective way. The spirit of the book is modern, for instead of being merely a list of the parasites inducing diseases, there is a chapter on the nature and symptoms of diseases, and another on the structure and functions of plants. The classification of the disease-producing fungi is restricted to a single chapter, and then a series of chapters takes up the study of the best known tropical diseases. Two final chapters discuss prevention and control, fungicides and spraying apparatus.—J. M. C.

A weed flora

PAMMEL[5] has set the pace for a comprehensive book on the weed flora of a state. He makes the statement that a conservative estimate of the damage done to the crops of Iowa by weeds is $25,000,000 annually. If this is true, it is certainly high time the farmers should learn to recognize the dangerous weeds and eliminate them. The contents of the volume can be best indicated by the chapter titles. The first chapter is a descriptive manual (400 pp.), in which every weed is illustrated and its distribution through the state indicated upon a map. The remaining chapter titles are as follows: the general character of seeds; the microscopic structure of some weed seeds; morphology of flowers and leaves; scattering of weeds; roots and rootstocks of weeds; number and kind of weeds in different soils; injuriousness of weeds; weed migration; medicinal weeds; phenology of weeds; weeds and seed laws.—J. M. C.

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[4] COOK, M. T., The diseases of tropical plants. 8vo. pp. xi+317. *figs.* 85. London: Macmillan. 1913. $2.75.

[5] PAMMEL, L. H., The weed flora of Iowa. Iowa Geological Survey. Bull. no. 4. pp. xiii+912. *figs.* 570. 1913.

MINOR NOTICES

Index Filicum.—In 1905 CARL CHRISTENSEN published his *Index Filicum*, and now a supplement has appeared,[6] covering the period 1906–1912. The two parts are (1) the supplement, which contains all the names of ferns published during 1906–1912, and (2) corrections. In the supplement there are enumerated 33 names of new genera and subgenera that have been proposed. The number of species described as new during the period covered, and adopted in the supplement, is 1644. Eliminating the older species that have been reduced to synonyms, and adding the new ones, the number of species of ferns recognized by CHRISTENSEN at the end of 1912 is 7411.—J. M. C.

The fresh-water flora of Germany, Austria, and Switzerland.—Part 14 of this series of brochures has now appeared.[7] The four previous parts have been noted in this journal.[8] The present part presents the Bryophytes, and the keys, descriptions, and excellent illustrations should make their identification comparatively easy. The genera and species presented under the three groups are as follows: Sphagnales, 48 species; Bryales, 50 genera and 131 species; Hepaticae, 25 genera and 60 species.—J. M. C.

NOTES FOR STUDENTS

Natural vegetation and crop production.—In a careful study in one of the broad valleys in the Great Salt Lake Basin, KEARNEY[9] and his associates have shown that the natural vegetation of the area is so reliable an indication of the physical and chemical conditions affecting plant life that it affords an excellent basis for estimating the capabilities of the land for crop production. The report gives a good example of the quantitative investigation of the moisture and salt contents of the soil and the efficiency of the wilting coefficient in expressing the relation of the soil moisture to plant life and growth. Nearly all of the plant associations considered are dominated by a single species and very definitely limited in their extent.

The sagebrush (*Artemisia tridentata*) association is found in the higher portions of the valley. With an average precipitation of 40 cm., the growth-water is exhausted early in the summer, as shown by their determinations, but there is no excess of alkali salts. A good development of this association

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[6] CHRISTENSEN, CARL, Index Filicum. Supplementum 1906–1912. pp. 132. Hafnia, Denmark: H. Hagerup. 1913.

[7] PASCHER, A., Die Süsswasser-Flora, Deutschlands, Österreichs, und der Schweiz. Part 14. Bryophyta, by C. H. WARNSTORF, W. MONKEMEYER, and V. SCHIFFNER. pp. 222. *figs. 158*. Jena: Gustav Fischer. 1914. *M* 5.60.

[8] BOT. GAZ. 56:233. 1913.

[9] KEARNEY, T. H., BRIGGS, L. J., SHANTZ, H. L., and others. Indicator significance of vegetation in Tooele Valley, Utah. Jour. Agric. Research 1:365–417. 1914.

indicates land well adapted to both dry and irrigation farming, the taller and thicker the sagebrush the richer the soil.

Areas next below the sagebrush are occupied by the *Kochia* (*K. vestita*) association, and this was found to indicate a soil of finer texture, with similar moisture conditions, but with only the first foot of soil free from an injurious quantity of alkali salts. Dry farming does not succeed on this area because of the small amount of soil free from alkali, while the relatively impervious nature of the soil prevents the washing out of the alkali by irrigation; hence *Kochia* land scarcely permits crop production.

Following the *Kochia* belt comes the shad scale (*Atriplex confertifolia*) association, with soil moisture and alkali conditions similar to the preceding, but with a soil containing much gravel. Here dry farming does not succeed, but with irrigation the alkali would be more readily washed out and hence the crop probabilities are better.

The study continues with the associations of the lower, more alkaline areas, indicating as before the agricultural possibilities of the land. In addition to the value of the various associations as crop indicators, their composition is detailed, the successions noted, and the root characters of the principal species studied. The report is an excellent example of applied ecology.—G. D. FULLER.

The ecology of Calluna.—The common European heather, *Calluna vulgaris*, has been regarded as a typical calciphobe by CONTEJEAN and other partisans of the chemical as opposed to the physical theory of plant distribution. Furthermore, the heather is regarded as partial to acid soils, which also are deficient in certain mineral constituents, and hence are betokened sterile. *Calluna* occurs in various places on the chalk downs of southern England, being accompanied by several other species that are alien to the ordinary flora of the chalk; usually the heather patches are sharply delimited from the surrounding chalk associations. Miss RAYNER[10] has been making some detailed investigations of *Calluna* on the downs, and although much remains to be explained, a large contribution has been made to the ecology of the species. On the downs the heather grows in neutral soil, rich in most ordinary mineral constituents, but poor in lime; the roots, however, often penetrate down into soil areas that are rich in lime. The proportion of magnesia is relatively high. Cultures were made in soil from the heather areas and in ordinary chalk soil containing over 40 per cent of calcium carbonate. In the latter soil there were noted certain abnormalities, such as reduced capacity for germination and arrested development of the various organs; extensive bacterial colonies developed about the roots, and the mycorhizal fungus developed poorly. The relation of *Calluna* to its root fungus is obligate; the primary root becomes infected

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[10] RAYNER, Miss M. C., The ecology of *Calluna vulgaris*. New Phytologist **12**: 59–77. *pl. 1. figs. 2.* 1913; see also preliminary paper, *ibid.* **10**: 227–240. *figs. 2.* 1911.

shortly after germination, the mycelium coming from the seed coat which is infected while still in the ovary. In sterile cultures there is a complete arrest of root development. It is concluded that the so-called soil preference of *Calluna* depends upon the maintenance of a biological balance between the roots and the root fungi. The disturbance of this balance in soils rich in lime is responsible for poor growth in such soils. Probably also the increased bacterial growth in the chalk cultures is detrimental. It is to be hoped that the author will make a comparative study of *Calluna* in its ordinary habitat, where the soil is acid and infertile.—H. C. COWLES.

A new Tylodendron.—WEISS[11] has described a new form of *Tylodendron* under the name *T. Cowardi*. Particular interest in this specimen centers around the secretory canals and primary wood. WEISS says: "The outer portions of the pith have very numerous secretory canals with dark brown contents, very like those found in the Medullosae, in cycads, and also in the pith of *Poroxylon*. These have not been observed or described, so far as I know, in any other specimens of *Tylodendron* showing structure." There are also isolated groups of primary xylem at the periphery of the pith as in *Pitys antiqua*, but much smaller. Analogous structure is seen in *Mesopitys* of ZALESSKY and in *Cycadeoidea* of WIELAND. With regard to the systematic position of this *Tylodendron*, WEISS agrees with POTONIÉ that *Tylodendron* is of araucarian affinity. He considers that the pitting of the secondary wood, the double leaf traces, etc., are araucarian or cordaitean in character, and homologizes the secretory ducts in the pith with those in the same region of the cone of *Araucaria* and the stem of *Poroxylon*. WEISS further considers that since a *Tylodendron* has recently been described with discoid pith like the Cordaiteae, considerable light is thrown on the connection between the cordaitean and the araucarian forms, and that the study of this genus promises much along this line in the future.—R. B. THOMSON.

Plant invasion on Hawaiian lava flows.—Thanks to the initiative of TREUB, we are now well informed as to the revegetation of Krakatau. The successive lava flows from Mauna Loa, the age of many of which is exactly known, afford an excellent opportunity for comparable investigations. The results of a preliminary study of this sort are given by FORBES.[12] As is well known, the lava here is of two well defined sorts, the *pahoehoe*, which has a smooth and satiny exterior and may be ropy, and the *aa*, which has a cavernous and jagged exterior. On a lava flow of 1859, no vascular plants were found on the *aa*, though the surface was often white with lichens. To FORBES' surprise,

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[11] WEISS, F. E., A *Tylodendron*-like fossil. Mem. Proc. Manchester Phil. Soc. **57**: pp. 14. *pls.* 2. 1913.

[12] FORBES, C. N., Preliminary observations concerning the plant invasion on some of the lava flows of Mauna Loa, Hawaii. Occasional papers of the Bernice Pauahi Bishop Museum of Polynesian Ethnology and Natural History **5**:15–23. 1912.

the smooth *pahoehoe* was much more richly covered with vegetation, which occurred, however, only in cracks. On a 1907 flow plants were found just beginning to be established. The author concludes that on both types of lava, the first pioneers are lower cryptogams; on the *pahoehoe* these are soon succeeded by ferns and seeds plants, but on the *aa* there is a long-enduring lichen stage. Ultimately the natural forest of the region returns, except in places where man's influence causes the successful invasion of a naturalized flora. The *ohia* (*Metrosideros polymorpha*) is the dominating tree at first, and the *koa* (*Acacia koa*) is the dominating tree of the ultimate or climax forest.—H. C. COWLES.

Rainfall and soil moisture.—In studying the conditions which govern the plant activities of the semi-arid region about the Desert Laboratory, Tucson, Arizona, SHREVE[13] has made weekly determinations of the soil moisture at depths of 3, 15, and 30 cm. throughout the year, and compared the resulting data with the record of the rainfall for the same period, in order to see exactly how the former is affected by the latter. It is evident that precipitation of less than 0.15 inch has no effect upon the soil moisture, and that therefore there are periods of 140 days in the region under consideration without rainfall of sufficient amount to increase the moisture in the soil. This serves to indicate that in desert regions by no means all of the small rainfall is significant to vegetation as a source of water supply. The evaporation has been determined and plotted along with its ratio to the soil moisture, the march of soil moisture throughout the year, and the distribution of rainfall, making an instructive and detailed chart of those moisture factors which affect vegetation. Among other things it proves the range of moisture conditions at the Desert Laboratory to be one of great extremes.—G. D. FULLER.

Drought resistance in Hopi maize.—For centuries the Indians of New Mexico and Arizona have grown a race of maize in soil that is much too dry for the ordinary races of the species. A large factor in the success of this race, known as Hopi maize (from the Hopi Indians), is the extraordinary capacity for elongation possessed by the mesocotyl.[14] The Indians are accustomed to plant their maize at a depth of 15–45 cm.; this depth is for most varieties too great for effective germination. In ordinary races the mesocotyl can rarely be forced to grow to a length greater than 10 cm., whereas a length of 36 cm. can be induced in Hopi maize. Another advantage in the mesocotyl of Hopi maize is its ability to produce roots, a rare phenomenon in grass internodes. A third feature of great importance is the great elongation of the primary root

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[13] SHREVE, F., Rainfall as a determinant of soil moisture. Plant World 17:9–26. *figs.* 3. 1914.

[14] COLLINS, G. N., A drought-resisting adaptation in seedlings of Hopi maize. Jour. Agric. Research 1:293–302. *figs.* 2. *pls.* 29–32. 1914.

in Hopi maize. However these striking features may have originated, it is obvious that they enable this race to grow in much more arid situations than other races of maize, and it is suggested that it may well become an important economic plant in arid regions.—H. C. COWLES.

Indiana Academy of Science.—The volume of *Proceedings* for 1912 contains the following abstracts and papers of botanical interest: "Further notes of the seedless fruits of the common persimmon (*Diospyros virginiana* L.)," and "The influence of certain environic factors on the development of fern prothallia," by DAVID M. MOTTIER; "The mosses of Monroe County," by F. L. PICKETT and MILDRED NOTHNAGEL; "Length of life of *Arisaema triphyllum* corms," and "Acetic alcohol as a killing and fixing agent in plant histology," by F. L. PICKETT; "Plants not hitherto reported from Indiana," by CHAS. C. DEAM; "Report of the work in corn-pollination, IV," by M. L. FISHER; "Conjugation in *Spirogyra*," by F. M. ANDREWS; "Photosynthesis in submerged land plants," by H. V. HEIMBERGER; "Indiana fungi, III," by J. M. VAN HOOK; "Fungous enemies of the sweet potato in Indiana," by C. A. LUDWIG; "Notes on some puff balls of Indiana," by FRANK D. KERN; "The improvement of medicinal plants," by F. A. MILLER; "The structure and diagnostic value of the starch grain," by R. B. HARVEY.—J. M. C.

Structure of tropical amphibious plants.—Material of *Ipomea reptans* and *Neptunia prostrata* obtained from pools in northwestern Madagascar that are quite dry during a considerable portion of the year was examined by CHOUX,[15] who compared the anatomy of the portions developed during the wet and dry seasons. He found considerable differences in size and external appearance, while in internal structure the stems developed during the dry season showed (1) proportionately greater development of vascular and fibrous tissue, together with smaller air passages; and (2) a considerable amount of stored starch, a food reserve quite lacking in portions developed during the humid season. It would seem, therefore, that in these two tropical amphibious forms the growth activity results during the dry season in the accumulation of reserve food; while during the wet season the growth is so vigorous that it uses not only the food then manufactured, but also that which has been accumulated during the previous months.—G. D. FULLER.

Osmosis in soils.—The recent results obtained by LYNDE,[16] showing that certain soils, notably the clays, promote the movement of soil water by acting as semipermeable membranes, increasing in efficiency with their depth, suggest

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[15] CHOUX, P., De l'influence de l'humidité et de la sécheresse sur la structure anatomique de deux plantes tropicales. Rev. Gén. Botanique **25**: 153–172. 1913.

[16] LYNDE, C. J., Osmosis in soils. Soils act as semipermeable membranes I. Jour. Phys. Chem. **16**: 759–765. 1912; and LYNDE, C. J., and BATES, F. W., Osmosis in soils. Soils act as semipermeable membranes II. *Ibid.*, 766–781.

several applications of the theory of interest to ecologists and agriculturalists. These applications are of all the more interest because the efficiency of the finer soils has been shown[17] to be such that very considerable pressures may develop, which must be important factors in the upward movement of water in certain soils. It follows that whatever affects an increase in the concentration of the soil solution in the upper strata of such soils, whether it be the application of fertilizers or evaporation or the action of soil bacteria, aids materially in increasing the amount of water raised through the subsoil. Such factors may be involved in the increased water supply in climax mesophytic plant associations.—G. D. FULLER.

Liassic flora of Mexico.—WIELAND[18] has published a further study of the Liassic flora of Mixteca Alta, Mexico. The most significant botanical results were published in this journal.[19] The present contribution consists of a study of the composition of the flora largely for stratigraphical purposes. WIELAND shows that aggregates of species enable one to determine the age of a formation on the basis of the percentage of the major elements of the flora. He gives a table of the flora considered, with relationships to other floras that have been reported. The most conspicuous feature of this aggregate is the dominance of cycadophytes, which constitute 70 per cent of the flora. He gives illustrations of the overlapping of floras in the Jurassic. The suggestion of using the composition of a whole flora rather than certain species selected arbitrarily in the determination of strata seems to be a good one.—J. M. C.

The vegetation of Gothland.—THOMAS[20] has given an account of the vegetation of the Swedish island, Gothland. The island has an area of 1220 square miles, about half of which has more or less primitive vegetation. Some plants rare on the mainland, as the walnut and ivy, are common here. THOMAS, using the terminology of the English ecologists, finds that about half of the island was originally fenland (the term fen, by the way, should be adopted by American ecologists, as we have no good equivalent name for this very common formation); the vegetation aspect here and the constituent species are much as in the English fens. Calcareous bogs exist, comparable to those of Northern England. Various successions terminating in forest can be readily worked out, and the author appeals for an intensive study before virgin conditions are altogether gone.—H. C. COWLES.

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[17] LYNDE, C. J., and DUPRÉ, H. A., On osmosis in soils. Jour. Amer. Soc. Agron. **5**:102–106. 1913.

[18] WIELAND, G. R., The Liassic flora of the Mixteca Alta of Mexico; its composition, age, and source. Am. Jour. Sci. **36**:251–281. *figs. 2*. 1913.

[19] BOT. GAZ. **48**:427–441. 1909.

[20] THOMAS, H. H., The vegetation of the island of Gothland. New Phytol. **10**:260–270. *pls. 2*. 1911.

Anatomy and plant hybrids.—Miss HOLDEN[21] has suggested that anatomical structures may be used in the recognition of spontaneous hybrids which are identical in external appearance with ordinary species. There can be no question but that spontaneous hybrids are of extremely common occurrence, and when they are recognized at all, they are described as variations of recognized species. She uses as illustration a case of identical external structure covering profound differences in internal organization. A hybrid between *Betula pumila* and *B. lenta* was recognized by anatomical structure which otherwise appeared to be *B. pumila*. Another illustration is obtained from a form of *Equisetum* which was clearly proved to be a hybrid. If this weapon proves to be as efficient as Miss HOLDEN hopes, it will go far toward attacking successfully the problem of mutations.—J. M. C.

An Arkansas prairie.—As a result of a tour of a portion of Eastern Arkansas, HARPER[22] gives us some notes upon the phytogeography of the region, the most interesting of which concern an area of natural prairie, known as Grand Prairie, in Prairie County. The plant list, made about the middle of June, shows a very rich flora, estimated at 150 species, in which the Compositae, Leguminosae, and Juncaceae are well represented. No solution is offered of the problem of the occurrence of a prairie in this old flood plain other than indications that the soil moisture shows great extremes when spring and midsummer conditions are contrasted. The study of such areas in Arkansas seems to have been neglected and to offer excellent opportunities for botanical investigation.—G. D. FULLER.

The vegetation of the Hempstead Plains.—R. M. HARPER presents the interesting floral features of the Hempstead Plains, which are situated in the western part of Long Island, New York.[23] In this area there is a natural prairie of some 50 square miles in extent, much of which shows vegetation essentially undisturbed by human influences. The commonest herb is *Andropogon scoparius*, which also is common on many western prairies. HARPER discusses the possible causes of such a prairie, without coming to definite conclusions, except that climatic theories are ruled out, as is the influence of fire or of grazing. It is more likely, he thinks, that the Hempstead prairie is associated with some peculiar type of soil.—H. C. COWLES.

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[21] HOLDEN, RUTH, Anatomy as a means of diagnosis of spontaneous plant hybrids. Science N.S. **38**:932, 933. 1913.

[22] HARPER, R. M., Phytogeographical notes on the coastal plain of Arkansas. Plant World **17**:36–48. 1914.

[23] HARPER, R. M., The Hempstead Plains; a natural prairie on Long Island. Bull. Amer. Geog. Soc. **43**:351–360. *figs.* 5. 1911; also Torreya **12**:277–286. *figs.* 7. 1912.

Symbiosis between algae and sponges.—Two new species of red algae have been described by Madame WEBER VAN BOSSE[24] as belonging to the genus *Thamnoclonium* and living in symbiosis with a sponge which forms a continuous layer over the flattened and branching thallus of the plants. In the sponge are found imbedded small branches of the algae and also a series of filaments that are apparently epidermal outgrowths of the plant, but whose nature and origin could not be definitely determined on account of the scantiness of the material which was collected on some East Indian islands. The character of the relationship between the two symbionts remains to be determined.—G. D. FULLER.

Flora of New Guinea.—A volume of the botanical results of the Dutch scientific exploration of New Guinea during 1912 and 1913 has appeared.[25] Previous parts of the botanical report on New Guinea were reviewed in this journal.[26] The present part includes the Orchidaceae by J. J. SMITH. The species number 151, included in 41 genera, 5 of the species being described as new. By far the largest genus is *Dendrobium*, with 52 species, *Bulbophyllum* being next with 24 species. The present contribution brings together previous publications of SMITH, who is credited with 130 of the species and 3 of the genera. In *Dendrobium*, SMITH has described 47 of the 52 species.

Another volume of the botanical results of the Dutch scientific exploration of New Guinea during 1907 and 1909 has also appeared.[27] The preceding part was published in 1912.[28] In the present part the collaborators are HANS HALLIER and TH. VALETON. Altogether 9 families are presented, all monocotyledons, including 25 genera and 113 species, 62 of which are new. Most of the contribution consists of the presentation of Zingiberaceae by VALETON, including 10 genera and 92 species (56 new). The large genera are *Alpinia*, with 35 species (22 new), and *Riedelia*, with 28 species (21 new).—J. M. C.

A toxin from Rhizopus.—BLAKESLEE and GORTNER[29] have announced the discovery of a toxin produced by *Rhizopus nigricans*. The expressed juice from aerial filaments caused almost instant death when injected intravenously into rabbits. Since this fungus has a very wide distribution, and is almost

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[24] WEBER VAN BOSSE, Madame A., Sur deux nouveaux cas de symbiose entre algues et éponges. Ann. Jard. Bot. Buitenzorg. Suppl. **32**[2]:587–594. 1910.

[25] Nova Guinea. Résultats de l'expédition scientifique Néerlandaise à la Nouvelle-Guinée en 1912 et 1913 sous les auspices de A. FRANSSEN HERDERSHEE. Vol. XII. Botanique. Livraison IV. 4to. pp. 1–108. *pls. 1–28*. Leide: E. J. Brill. 1913.

[26] BOT. GAZ. **49**:464. 1910; also **55**:462. 1913.

[27] Nova Guinea. Résultats de l'expédition scientifique Néerlandaise à la Nouvelle-Guinée en 1907 et 1909 sous les auspices de Dr. H. A. LORENTZ. Vol. VIII. Botanique. Livraison V. 4to. pp. 899–988. *pls. 160–179*. Leide: E. J. Brill. 1913.

[28] BOT. GAZ. **55**:462. 1913.

[29] BLAKESLEE, A. F., and GORTNER, ROSS AIKEN, On the occurrence of a toxin in juice expressed from the bread mould, *Rhizopus nigricans* (*Mucor stolonifer*). Biochem. Bull. **2**:542–544. 1913.

certain to infect starchy food under suitable moisture conditions, the suspicion is suggested that it may be related to certain destructive diseases of stock, such as pellagra ("corn-stalk disease"). Experiments are being conducted to discover the nature of the toxin and its possible relation to such diseases.—J. M. C.

A new form of Juglans.—BABCOCK[30] has investigated a new form of *Juglans californica* and described it as var. *quercina*, on account of the resemblance of its leaves to those of an oak. The new form has appeared on seven separate occasions among seedlings of at least three different trees of *J. californica*. Three working hypotheses were tested experimentally, the conclusions being that the form is not a hybrid of *J. californica* with *Quercus agrifolia* or some other oak; that it did not originate in certain teratological flowers that occur; and that in all probability it is a mutant.—J. M. C.

Vascular anatomy of Platycerium.—Miss ALLISON[31] has investigated the vascular anatomy of the rootstock of three species of *Platycerium*, uncovering a very unexpected complexity. The vascular cylinder is a complicated polystele, and in the largest form studied (*P. aethiopicum*) there are medullary strands also. She concludes that in the genus there is a progression from a comparatively simple type to a more complicated one. This anatomical structure certainly suggests a comparison with the Marattiaceae and the *Pteris*-like forms.—J. M. C.

Mosses of New Zealand.—DIXON[32] has begun a publication of a series of studies of the mosses of New Zealand, especially with reference to the herbarium of ROBERT BROWN at Christchurch. The first part contains a revision of the species of *Dicranoloma*, 16 species being recognized, 5 of which are described as new. These species have heretofore been included under *Dicranum*, and DIXON follows RENAULD'S treatment of this group as a separate genus.—J. M. C.

Medullosa pusilla.—In his *Studies in fossil botany* (1909), SCOTT referred to a very small *Medullosa* closely resembling the well known *M. anglica* except in size. He named it provisionally *M. pusilla*, and now has given a further account, with illustrations.[33] Further study shows that it differs in no important respect from *M. anglica*, and that its chief interest probably lies in the fact that it is the smallest *Medullosa* on record.—J. M. C.

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[30] BABCOCK, ERNEST B., Studies in *Juglans* I: Study of a new form of *Juglans californica* Watson. Univ. Calif. Publ. Agric. Sci. **2**:1–46. *pls. 1–12.* 1913.

[31] ALLISON, HARRIET E., On the vascular anatomy of the rhizome of *Platycerium*. New. Phytol. **12**:311–321. *figs. 5.* 1913.

[32] DIXON, H. N., Studies in the bryology of New Zealand, with special reference to the herbarium of Robert Brown. Part I. New Zealand Inst. Bull. no. 3. pp. 29. *pls. 1–4.* 1913.

[33] SCOTT, D. H., On *Medullosa pusilla*. Proc. Roy. Soc. London B **87**:221–228. *pl. 13. figs. 2.* 1914.

Embryo of Helminthostachys.—LANG[34] has supplied some much needed information in reference to the embryogeny of *Helminthostachys*. In a preliminary note[35] he had announced the existence of a well developed suspensor, but the present study furnishes many additional details. The embryo extends down into the prothallium before segmentation takes place, and the first two walls are transverse. The cell next to the neck of the archegonium, which may divide or not, forms the upper suspensor tier; the middle cell, which divides, forms the second suspensor tier; while the terminal cell forms the embryo proper. The hypobasal half of the embryo forms the foot, while from the epibasal half the stem tip, first leaf, and probably the first root arise. A comparative study of the embryogeny of Marattiaceae, Ophioglossaceae, and seed plants leads to the suggestion that "the suspensor represents the last trace of the filamentous juvenile state in development of the plant, and may have persisted in the seed-plants from their filicineous ancestry."—J. M. C.

Basidiomycetes of the Philippines.—GRAFF[36] has published a list of additions to the known Basidiomycetes of the Philippines with descriptions of new species. These additions number 33, about equally distributed between Hymenomycetes and Gasteromycetes. The new species are described in *Exidia*, *Laschia*, *Lentinus*, *Volvaria*, *Naucoria*, and *Bovista*.—J. M. C.

Nectaries and phylogeny.—After examining the nectaries of a large number of monocotyledons and dicotyledons, PORSCH[37] reaches the conclusion that the nectary is not only an organ of some phylogenetic significance, but that it furnishes additional proof of the derivation of the former from the latter.—C. J. CHAMBERLAIN.

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[34] LANG, WILLIAM H., Studies in the morphology and anatomy of the Ophioglossaceae. II. On the embryo of *Helminthostachys*. Ann. Botany **28**:19–37. *figs. 9. pl. 3.* 1914.

[35] Ann. Botany **24**:611. 1910.

[36] GRAFF, P. W., Additions to the basidiomycetous flora of the Philippines. Philippine Jour. Sci. **8**:299–307. *pls. 8–10.* 1913.

[37] PORSCH, OTTO, Die Abstammung der Monokotylen und die Blütennektarien. Ber. Deutsch. Bot. Gesells. **31**:580–590. 1914.

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THE PROBABLE ORIGIN OF OENOTHERA LAMARCKIANA SER. (WITH PLATES XVII–XIX). *Hugo De Vries* - - -

VOLUME LVII NUMBER 5

THE
BOTANICAL GAZETTE

MAY. 1914

THE PROBABLE ORIGIN OF OENOTHERA LAMARCKIANA SER.

HUGO DE VRIES

(WITH PLATES XVII–XIX)

In a series of most interesting articles, B. M. DAVIS has recently tried to prove that mutability might be a result of previous crosses. This view was first proposed by BATESON and SAUNDERS, and applies especially to the phenomena which *Oenothera Lamarckiana* shows when seeds from the pure strain, and even from pure lines within this strain, are sown, as in the experiments I conducted in my experimental garden. DAVIS expected to be able to offer the desired proof by showing that *O. Lamarckiana* might be duplicated by crossing two other species of the same group. Up to this time, as a matter of fact, he has not succeeded in producing any form which comes sufficiently near *O. Lamarckiana* to be compared with it.[1] But if he had succeeded in doing so, evidently it would not have been a proof for his assertion, unless his hybrid should show the same degree of mutability as does *O. Lamarckiana*, since we have as yet no means of judging from the morphological characters of a given plant whether its hereditary characters are in a stable or in an unstable condition.

In starting his experiments to produce a duplication of LAMARCK'S evening primrose, DAVIS was unfortunate in the choice of the species for his combination. He chose *O. biennis* L. and a

[1] For a successful duplication of an elementary species by means of crossing, see *Oenothera biennis*×*O. cruciata* Nutt. in Gruppenweise Artbildung, p. 311.

form which he assumed to be *O. grandiflora* Aiton. It is evident that the first condition of success in such work consists in the purity and the immutability of the species which are to produce the hybrid. If they are already in a mutable condition, it is to be expected that their hybrids, or at least some of them, may combine the different lines of mutability of their parents; and at all events, the mutability of such a hybrid would be no proof that this phenomenon may be produced by means of crossing. On the other hand, if the species to be crossed, or even only one of them, were not pure, the hybrid might inherit this impurity and show phenomena which might easily be mistaken for mutations.

It so happens that *O. biennis* is in a condition of mutability analogous to that of *O. Lamarckiana*, although not developed to the same high degree. From time to time it produces dwarfs, which are distinguished from it by exactly the same two characters which differentiate the dwarfs of *O. Lamarckiana* from their mother species, namely, low stature and sensitiveness to the attacks of some species of soil bacteria.[2] Moreover, STOMPS has shown that *O. biennis* may, although very rarely, double the number of chromosomes in its sexual cells, which in *O. Lamarckiana* produces the two mutants *O. gigas* and *O. semigigas*.[3] As is now generally admitted, *O. gigas* results from the pairing of two mutated sexual cells, each of which had a double number of chromosomes. *O. semigigas*, on the other hand, is produced by the pairing of a sexual cell mutated in the same way, with a normal gamete; therefore it possesses only 21 chromosomes (14+7), while the number in *O. gigas* is 28. As yet, only *semigigas* mutants have been observed coming from *O. biennis*, and it is obvious that the double combination must be much rarer. As a proof of this special kind of mutability in *O. biennis*, however, the observations of STOMPS are wholly sufficient.

In quoting these facts, DAVIS says that if it can be shown "that tested strains of this *biennis* are able to produce new forms of specific

[2] STOMPS, TH. J., Mutation von *Oenothera biennis* L. Biol. Centralbl. 32:521–535. 1912; also ZEYLSTRA, H. H., *Oenothera nanella* De Vries, eine krankhafte Pflanzenart. Biol. Centralbl. 31:129–138. 1911. Vergl. ferner: Gruppenweise Artbildung 1913:296–304.

[3] STOMPS, TH. J., *op. cit.* p. 533.

rank or even marked varieties, the mutationists would have much stronger evidence in support of the mutation theory than that based on the behavior of *O. Lamarckiana*."[4] After conceding this strong position to his adversaries, DAVIS subjects the results of STOMPS to a rather sharp criticism, which, unfortunately, is based upon a confusion of two wholly distinct types, namely, *O. biennis* L. var. *cruciata*[5] and *O. cruciata* Nutt. He says: "It should be made clear that the form (*O. biennis cruciata*) is recognized in the more recent taxonomic treatments as a true species sharply distinguished from types of *biennis* by its floral characters," and "a cross between these types must certainly be regarded as a cross between two very distinct evolutionary lines and its product as a hybrid in which marked modifications of germinal constitution are to be expected."[6] But, as a matter of fact, the Dutch *O. biennis cruciata* differs from *O. biennis* only in the characters of the petals; in all other respects it is wholly the same, and therefore evidently only a subordinate variety of this species. It has not been dealt with in recent taxonomic treatments, since it occurs almost exclusively in the sand dunes of Holland, where it is produced from time to time by mutation from the mother form (first observed in 1900), without having been able until recently to multiply in the field so as to produce a persistent local variety.[7]

On the other hand, *O. cruciata* Nutt. is quite a different species, with narrow, brownish green leaves, and a different type of branching, of spikes, and of fruits. It grows wild in New York and Vermont, and is well known to all students of the American flora. By some authors it has been considered a variety of *O. biennis*, and this probably is the chief cause of DAVIS' confusion. The character and the behavior of its hybrids with *O. biennis* have been amply dealt with in my *Gruppenweise Artbildung*.

In the experiment of STOMPS, the dwarf and *semigigas* mutations were produced by hybrid strains of *O. biennis* and *O. biennis*

[4] DAVIS, B. M., Mutations in *Oenothera biennis* L.? Amer. Nat. **47**:116–121 (especially p. 116). 1913; see also *op. cit.* **47**:540–596 (especially p. 567). 1913.

[5] Die Mutations-Theorie **2**:599. 1903.

[6] Amer. Nat. **47**:117. 1913.

[7] Die Mutations-Theorie 2:599. 1903.

cruciata, and it was assumed that such strains would behave as true species in all characters not related to the differentiating marks of the petals. It must be conceded, therefore, that the cross of these two forms may be treated "as though it were the combination of forms within the same species, which have similar germinal constitutions" (DAVIS, *op. cit.* p. 117).

But the most clear and simple way of obviating this whole objection is evidently to sow seeds of *O. biennis* of pure descent upon the same large scale as in the former experiment. This has been done, and a dwarf and a *semigigas* form have been produced by this pure line, besides some other mutations.[8] They had the same characters as the former ones, and now provide us with the "strong support" asked for by DAVIS. Moreover, they show that his choice of *O. biennis* for a proof of the assertion that mutability might be produced by crossing immutable species was a most unhappy one.

The second condition for success in this kind of work is, as has been stated, the purity of the types to be crossed. As already quoted, DAVIS assumes that a cross between two very distinct evolutionary lines may give a hybrid with marked modifications of germinal constitution. This may be applied to his choice of the type which he calls *O. grandiflora*, and which he has made the other parent of his initial cross. He got his seeds from Dixie Landing, Alabama, a locality where BARTRAM had discovered *O. grandiflora* about a century ago. He assumed them to be of the pure species, but a culture which I made in my garden from seeds kindly supplied to me by Mr. DAVIS proved to be a mixture, and thereby threw a distinct doubt upon the purity of the station. For this reason I visited Dixie Landing in September 1912, and had the good fortune to be accompanied by Mr. H. H. BARTLETT, of Washington, well known for his systematic researches among the wild species of this group. We found the station in a most desolate condition. A small-flowered species, *O. Tracyi*, in almost all respects different from *O. grandiflora*, had migrated into the same old cotton fields and mixed everywhere with the species of BAR-

[8] STOMPS, TH. J., Parallele Mutationen bei den Oenotheren. Ber. Deutsch. Bot. Gesells. 30:Heft 3, 1914.

TRAM.[9] On no single field was the original form pure; it was always mixed to such a degree with *O. Tracyi* and with their hybrids that we found it impossible to collect undoubtedly pure *grandiflora* seed from this locality. Moreover, the intermediate types were so numerous (over a dozen) that it was difficult to regard all of them as normal hybrids between only two parents. To produce such a diversity of forms, either one or both of the parents must have been in a mutating condition, or more than two species must have combined in the crosses. In both cases, the material can hardly be considered as a fit starting-point for experiments bearing upon the causal relations of crossing and mutability.

Recently I have shown that besides *O. biennis* some other species of *Oenothera* are actually in a state of mutability, and especially has one of the most common American types thrown off marked mutants in my experiment garden.[10] The degrees of development of this condition, however, are very different in different species. In some of them mutations occur rarely, but they serve to throw a doubt upon the stability of those forms for which no positive results have as yet been won. In other words, we may say that almost all the nearest allies of *O. Lamarckiana* are open to the suspicion of sharing at least some degree of the mutability of this species. There is no use, therefore, in trying to produce mutability by crosses of species of the same subgenus (*Onagra*) in order to show that this phenomenon is only a result of crossing, as is asserted by DAVIS.

Moreover, I might point out that the question should be dealt with from a general standpoint and not be limited to the evening primroses. If it should be true that phenomena like those of *O. Lamarckiana* could be produced by crossing immutable species, it would, of course, be of much higher scientific value to produce them in other families or genera, or at least in the other subgenera of the evening primroses. The chance of finding immutable parents for a cross would be far greater and the proof could be given as easily and in many cases with less amount of mechanical work

[9] DE VRIES, HUGO, and BARTLETT, H. H., The evening primroses of Dixie Landing, Alabama. Science N.S. **35**:599–601. 1912.

[10] Gruppenweise Artbildung, pp. 296–312. 1913.

and space in the garden. The line of work chosen by DAVIS seems to me to be necessarily without any chance of success.

Besides his experimental work, DAVIS has made some historical researches to discover the origin of *O. Lamarckiana*.[11] Unfortunately, he has neglected to visit the Museum d'Histoire Naturelle at Paris, where the herbarium of LAMARCK is preserved, and where other valuable documents concerning the first appearance of our species are to be found. For myself I visited these collections in 1895 and reported on the results of my investigations in my *Mutation theory* (vol. I. pp. 437–444 of the English edition). In October 1913 I repeated my visit and compared the authentic specimens with the remarks made upon them by DAVIS. I regret to say that, through his ignorance of the available evidence, DAVIS has been led to conclusions which are fully contradicted by the herbarium material, both of the "Herbier de Lamarck" and of the "Herbier général" of the Museum. As we shall see, the origin of *O. Lamarckiana* is the same as I have pointed out in my book.

In the herbarium of LAMARCK, *O. grandiflora* (Lam.), which later was renamed by SERINGE and called *O. Lamarckiana*, the name it still bears, is represented by two large flowering specimens. When I studied them in 1895, they were loose on their sheets and bore together the no. 12, indicating that they corresponded with no. 12 *O. grandiflora* of the *Encyclopédie méthodique, Botanique*, by LAMARCK.[12] About 1900 they were fastened on new sheets and the numbers have been lost.[13] For convenience, I shall call these specimens *A* and *B*, the former being represented by our pl. XVII, while a photograph of *B* has been published by DAVIS.[14]

[11] DAVIS, B. M., Was LAMARCK'S evening primrose (*Oenothera Lamarckiana* Seringe) a form of *Oenothera grandiflora* Solander? Bull. Torr. Bot. Club **39**:519–533. *pls. 37–39*. 1912; A much desired *Oenothera*. Plant World **16**:145–153. 1913; The problem of the origin of *Oenothera Lamarckiana*. New. Phytol. **12**:233–241. 1913.

[12] The Mutation Theory **1**:442. 1901.

[13] The herbarium of LAMARCK was acquired by the Museum d'Histoire Naturelle in 1886. Vergl. BONNET, ED., L'herbier de LAMARCK, son histoire, ses vicissitudes, son état actuel. Jour. Botanique **16**:129–138. 1902.

[14] DAVIS, B. M., Was LAMARCK'S evening primrose (*Oenothera Lamarckiana* Seringe) a form of *Oenothera grandiflora* Solander? Bull. Torr. Bot. Club **39**:519–533. 1912. See *pl. 37*.

Unfortunately, these two specimens do not belong to the same elementary species, but the question as to which of them is to be considered as the authentic specimen has been answered by all authors in the same way, with the exception of DAVIS. According to the general agreement, *A* (pl. XVII) is the type of the species. DAVIS has not seen this specimen, and has based his judgment upon the communications of botanists concerned with systematic rather than with elementary species.

The plant *A* corresponds exactly with *O. Lamarckiana* Ser. as it is now universally cultivated and as I know it from my own cultures. The specimen is evidently a side branch, picked in the autumn, and the flowers, although very large, are not quite so large as may be seen in July and August. It bears no fruits, but the sexual organs of the flowers and the form of the flower buds do not leave the least doubt concerning its identity. The stigma lobes are widely spread and raised by the long style high above the tops of the anthers, and this is one of the best characters of *O. Lamarckiana*. The buds are conical and thick, and not thin as in *O. grandiflora* Ait. For comparison, I have given a group of flower buds (pl. XVII), picked in the autumn also, from my pure cultures. All the other marks correspond to those of the present species, although of course not all of them distinguish it from allied forms.

This sheet bears the label, "d'Amérique sept., tige rameuse, haute de 3 à 4 pieds," in the handwriting of LAMARCK. The description in the *Encyclopédie* says of the origin of the species: "Cette espèce est originaire de l'Amérique septentrionale. On la cultive au jardin du Muséum d'Histoire Naturelle (V.S.)."[15] The description, however, quotes some points which are not visible on the herbarium specimen, nor on specimen *B*. It is therefore clear that the author knew his plants from another source still, probably from the living material of the Jardin des Plantes. The most interesting point for us is the description of the fruits: "Le fruit est une capsule courte, cylindrique, glabre, tronquée légèrement, quadrangulaire, n'ayant environ que le tiers de la longueur

[15] V.S. ("vidi siccum") means that the diagnosis is based on herbarium material.

du tube calicinal."[16] This description wholly agrees with the fruits of the present species, especially if we remember that LAMARCK based his description on a comparison with the only other large-flowered form he knew, *O. longiflora*. The short fruits at once distinguish our species from the allied types, such as *O. suaveolens* Desf. and *O. grandiflora* Ait., which have thin and proportionally long fruits.[17]

This character of the fruits shows that the description of the *Encyclopédie* has been based upon specimen *A* and not upon the other one. For, although *B* lacks fruits also, it belongs to an elementary species which has long and narrow fruits, as we shall soon see. Here I might point out that in systematic researches of this kind, more value is to be attached to published diagnoses and descriptions than to the material preserved in a herbarium. The older systematists, as a rule, did not take much care of their material, even if they were very careful of their descriptions.[18] The herbarium specimens are often found without their names and without any indication concerning their origin. The rule "descriptio praestat herbario" applies in our special case, even as it does in many others. In our case, the description is relatively complete and clear, while in the dried specimen only part of the characters are represented.

For all these reasons I cannot agree with DAVIS, who says (p. 519) that I made an incorrect determination of the material of my cultures, when I identified it with LAMARCK's plant of 1796. The authentic specimen of LAMARCK and the description in the

[16] Encyclopédie méthodique, Botanique par LAMARCK, Tome IV, 1796. pp. 550–554, "Onagraire." Twelve species of this genus are enumerated, *O. longiflora* being no. 4, *O. corymbosa* no. 11, and *O. grandiflora* no. 12. A copy of the diagnosis of this last one may be found in my *Mutation theory* (p. 441) and in the article of DAVIS. The article in the *Encyclopédie* is not signed and was probably written by POIRET, who prepared many articles in vol. IV, and wrote the whole of the later volumes. In the herbarium of Paris some of the specimens may be seen quoted with the authority of POIRET, as, for example, on the sheet of *O. suaveolens* Desf., where above that name is written *Oenothera grandiflora* Poiret Encyclopédie. (Cf. *pl. 39* of the article of DAVIS.)

[17] L'*Oenothera grandiflora* de l'herbier de LAMARCK. Rev. Gén. Botanique 25: 1914.

[18] Cf. BONNETT, *op. cit.* p. 138.

Encyclopédie correspond as closely with the characters of my plants as dried specimens and descriptions expressed in words ever can do.

On the contrary, the specimen *B* is surrounded with doubts. DAVIS has given a very elaborate description of this branch, comparing it with my *Lamarckiana*. The sheet bears the label: "*Oenothera* . . . . [*grandiflora*] . . . . nova spec. flores magni lutei, odore grato, caulis 3 pedalis." The fact that the name *grandiflora* is placed in brackets shows that LAMARCK did not wholly trust his identification of this plant with the other one. Perhaps the words "nova species" indicate that he took it to be possibly a different species. Later, POIRET discovered the identity of this specimen with *O. grandiflora* Aiton Hort. Kew,[19] as has been indicated by DAVIS. And in DE CANDOLLE'S *Prodromus* (3:47. 1828), SERINGE separated the two types, describing *O. grandiflora* Ait. and *O. Lamarckiana* (SER. MSS) as different species.

The words "odore grato" point to *O. grandiflora* Ait., which has fragrant flowers, while the flowers of *O. Lamarckiana* Ser. are almost without odor. In the original description no mention is made of the odor, and this shows once more that the specimen *B* was not the authentic one for this description.

DAVIS has compared the branch *B* with some of his hybrid strains from Dixie Landing[20] and finds a close resemblance. Perhaps the plant of LAMARCK was a chance hybrid found in the Jardin des Plantes, and in this case, as DAVIS says, "we can have no certainty as to the characters of an individual plant unless its seeds have been grown in large cultures.[21] At all events, it is not backed by other herbarium material in the Museum d'Histoire Naturelle, so far as I know. If POIRET'S opinion that it belongs to *O. grandiflora* Ait. is correct, then it has evidently not served as a basis for the description of *O. grandiflora* Lam. (*O. Lamarckiana* Ser.). In *O. grandiflora* the fruits are thin and relatively large, for example,

[19] Encyclopédie méthodique. Suppl. IV, p. 141. 1816. See DAVIS, p. 522.

[20] At Dixie Landing, Alabama, only hybrid strains of *O. grandiflora* and *O. Tracyi*, perhaps mixed with other species too, are to be found. See Science *op. cit.* p. 399. 1912.

[21] DAVIS, B. M., A much desired *Oenothera*. Plant World 16:148. 1913.

3 cm. long and 3 mm. wide; while those of *O. Lamarckiana* may measure 2.5 cm. in length and 6 mm. in width, making a ratio of $\frac{10}{1}$ in the one case and $\frac{4}{1}$ in the other.[22] The description of the fruits as short, as given by LAMARCK, evidently points to the second and not to the first case.[23]

Summing up the main results of this discussion, we find that specimen *A* of the herbarium of LAMARCK closely corresponds to the *O. Lamarckiana* Ser. of the present time, and has been taken by almost all authors for its prototype. The specimen *B* differs from it in its general aspect, in the words "odore grato" on its label, and in the opinion of POIRET that it belongs to *O. grandiflora* Ait., this opinion pointing to long and narrow fruits. Personally, it impressed me as having been brought into the herbarium of LAMARCK only later on, and as having been placed in the cover of *O. grandiflora* Lam. with a doubt shown by the placing of the name in brackets.

The best proof for the fact that *A* and not *B* is the authentic specimen of *O. grandiflora* Lam. is perhaps given by the specimen in the herbarium of Father POURRET, which was given to the Muséum d'Histoire Naturelle by Dr. BARBIER in 1847.[24] It bears the name *Oenothera grandiflora* Lam. written in the clear and beautiful handwriting of the clerk of POURRET. In the same cover there is another sheet of POURRET'S collection, on which the same clerk wrote *Oenothera biennis*. Unfortunately, DAVIS, who did not visit the Museum, has mistaken this one for the one studied by me,[25] and has accordingly published a photograph (*pl. 38*) and a description of it. It is easily seen that this specimen really comes nearer to our present *O. biennis* L. than to anything else.

[22] L'*Oenothera grandiflora* de l'herbier de LAMARCK, *op. cit. fig. 1, b* and *c*.

[23] DAVIS (*op. cit.* p. 523) lays great stress on the tips of the sepals, but I cannot find a well defined difference between the two species in this character. He calls attention to the word "sétacé" in LAMARCK'S description of the sepal tips: "this has been translated by DE VRIES (*Mutations-Theorie*, p. 317. 1901) as "dicke." The French, however, is from the late Latin word *setaceus*, derived from "seta," a stiff hair or bristle. The meaning, therefore, is exactly the opposite of that given by DE VRIES." If the reader will kindly look up my book at the page quoted by DAVIS, he will find that I have translated "sétacé" by "fadenförmig."

[24] The Mutation Theory, Engl. ed. 1:442, note 2.

[25] Bull. Torr. Bot. Club *op. cit.* p. 527.

The plant which POURRET called *O. grandiflora* Lam. is represented on our pl. XVIII. It agrees wholly with the present *O. Lamarckiana* Ser., and in all respects. It was fastened on its sheet by the clerk of POURRET and consists of two flowering spikes and two separate flowers. The stigma lobes are seen spread above the anthers in the normal way. The specimens were picked at the beginning of the flowering period and bear no fruits; obviously they were main spikes. They will be recognized at once as *O. Lamarckiana* by anyone who has seen living cultures of this species. As I have quoted in my *Mutation theory* (*loc. cit.*), SPACH has written on this sheet "*Onagra vulgaris grandiflora* Spach," which remark also proves the identity with *O. Lamarckiana* Ser. The printed label says "Collection de l'Abbé POURRET, extraite de l'herbier légué par M. le Dr. BARBIER en 1847." The main spike measures about 40 cm., the smaller one about 20 cm.

In my book I have also referred to a specimen of *O. suaveolens* Desf. At that time I did not know the Alabama species and believed that *O. suaveolens* Desf. and *O. grandiflora* Ait. were synonyms, as almost all authors did. Therefore I used the two names promiscuously. Last summer, however, I cultivated, side by side, *O. suaveolens* Desf. from Fontainebleau, collected by Dr. BLARINGHEM, and *O. grandiflora* Ait. from Castleberry, Alabama, collected by myself with Mr. BARTLETT. They proved to be wholly different species.[26] So far as I know, the large-flowered Oenotheras, which are now relatively common in the western departments of France, all belong to *O. suaveolens* Desf., at least all the specimens and cultures on which I based my opinion in 1901 did. The specimen of the Muséum d'Histoire Naturelle, which I referred to especially, has been described by DAVIS from a photograph which is reproduced on *pl. 39* of his paper. DAVIS, who did not know the *O. suaveolens* as a separate species, called it the flotsam of the herbarium (p. 529); it is, on the contrary, the authentic specimen of DESFONTAINES, bearing on the label the name *suaveolens* written by DESFONTAINES himself. The smaller plant, fastened on the same sheet, has another label, saying only *O. grandiflora*, and seems to me to have been fastened on this sheet subsequently. The

[26] L'*Oenothera grandiflora* de l'herbier de LAMARCK, *loc. cit.*

larger one, however, corresponds exactly with the species which is now growing in many thousands of specimens near Samois on the eastern limit of the Forêt de Fontainebleau, where I visited the different stations with Dr. BLARINGHEM in October 1913. The long fruits and the thick flower buds do not leave the least doubt concerning the identity of this specimen.

The most interesting discovery in this field of historical research, however, is that of a specimen of *O. Lamarckiana* Ser. in the collection of MICHAUX, described recently by BLARINGHEM.[27] I had the advantage of studying this sheet myself, when I visited Paris in October 1913. The printed label says "Herb. Mus. Paris, Herbier de l'Amérique septentrionale d'ANDRÉ MICHAUX." There is no further indication of the locality and no name. The specimen is a main spike, picked in the beginning of the flowering period, and without fruits (pl. XIX). It is excellently preserved and corresponds in all respects to my cultures of *O. Lamarckiana* Ser. The lobes of the stigma are seen to be widely spread above the anthers. The flowers and flower buds are exactly those of the present species.

ANDRÉ MICHAUX died in 1802, after having traveled during twelve years through the eastern United States from the Hudson River to Carolina. His celebrated collection constitutes one of the best sources of our knowledge of the flora of those parts of America at the end of the eighteenth century, that is, of the same period in which LAMARCK published his volumes of the *Encyclopédie*. His herbarium is at present at the Muséum d'Histoire Naturelle at Paris, and his plants were described after his death by his son FRANÇOIS ANDRÉ MICHAUX in a book entitled "ANDRAEAS MICHAUX, Flora boreali-americana, sistens characteres plantarum quas in America septentrionali collegit ANDRAEAS MICHAUX."[28] MICHAUX had the habit of collecting seeds of as many species as possible, besides his herbarium specimens, and of sending them to Europe to be sown.

[27] BLARINGHEM, L., L'*Oenothera Lamarckiana* Seringe et les Oenothères de Fontainebleau. Rev. Gén. Botanique **25**:1914.

[28] Editio nova, 1820, Paris. The genus *Oenothera* is dealt with in vol. I on p. 214; the plant is given under the name of *O. biennis*. For the ground covered by his travels, see the preface and the article of BLARINGHEM.

This beautiful specimen proves that *O. Lamarckiana* Ser. was a component of the flora of the eastern part of Northern America at the end of the eighteenth century, and that it has come down to us as completely unaltered as may be shown by old herbarium specimens. Moreover, it tends to make it at least very probable that the European strains, or at least some of them, are derived from the importation of seeds by MICHAUX. The specimen *A* in the herbarium of LAMARCK, designated as "d'Amérique sept.," probably belonged to this same strain.

The exact situation of the locality where MICHAUX collected this specimen is, of course, unknown. Much stress is laid by many authors upon the fact that no wild station for *O. Lamarckiana* has been discovered lately in any part of the United States. This argument evidently loses the main part of its weight when we know that it was observed by such a well known botanist as MICHAUX. Moreover, this situation is not peculiar to *O. Lamarckiana;* on the contrary, the same condition prevails for the other European species, *O. biennis* L., *O. muricata* L., and *O. suaveolens* Desf., whose original stations in the United States and Canada have not been rediscovered. Even *O. grandiflora*, which is known to occur in Alabama in different localities, is observed there to grow on cultivated soil only, especially on old fields of corn and cotton, and no one knows whence it came. Therefore, if our present ignorance of the origin of *O. Lamarckiana* is adduced in order to throw a doubt on its reality as a good species, the same doubt is attached to its nearest allies, and, in fact, to all the dozens of elementary species of the group *Onagra* which are now being found wild on waste fields and along roadsides all through the United States. Autochthonous stations are not known for any of them.

A most valuable contribution to the clearance of the historical data concerning the origin of *O. Lamarckiana* Ser. has been brought forward by DAVIS in his criticism of the alleged Texan origin of the present cultivated strain. This was introduced into the trade by Messrs. Carter and Co. of High Holborn in the neighborhood of London, about the middle of the last century. These horticulturists offered the seeds as coming from Texas. But, since then, no botanist is known to have seen the plant in that state, and DAVIS

suggests (p. 523) that the statement might, perhaps, have been caused by a mistake.[29] Now, it is well known that such details are, as a rule, given more in the interest of advertising than in that of pure science. Moreover, no horticulturist likes to offer for sale seeds with the announcement that the same form may be found as a wild flower in his own country.

O. Lamarckiana has been, for many years at least, a component of the flora of England, growing in many localities, especially on the sand dunes along the coast. The most universally known station is that of St. Anne's on the Sea, near Liverpool, which has been studied by BAILEY, GATES, and other botanists, and where the species occurs in thousands of specimens. DAVIS received seeds from different English stations and recognized the plant in the cultures derived from them (*op. cit.* p. 237). In Lancashire the species locally grows together with *O. biennis* L., exactly as it does in the sand dunes of Holland. In such cases it produces hybrids such as I have described under the names of *laeta* and *velutina*, and as DAVIS has isolated as small-flowered races from those English localities (p. 237).

Now, if we agree with DAVIS that the seeds of Carter and Co. were derived from some English station, the probability at once arises that these English stations themselves owe their origin to the introduction of seeds from America, either by MICHAUX himself or by some other botanist of the same period. The history of the species would then become a very simple and clear one. In this respect it becomes of interest to look at the figure published in 1807 in SMITH'S *English Botany* (vol. VI. *pl. 1534*).[30] According to the description accompanying this plate, the "specimen was gathered on the extensive and dreary sand banks on the coast a few miles north of Liverpool, where millions of the same species have been observed by Dr. BOSTOCK and Mr. JOHN SHEPHERD growing perfectly wild and covering large tracts between the first and second range of sand hills." In this same locality *O. biennis* L. and *O. Lamarckiana* are now growing in the same abundance of individuals, partly separated and pure in different valleys and

[29] See DAVIS in New Phytol. 12:234. 1913.

[30] Cf. DAVIS, *op. cit.* p. 532.

partly in mixtures which are known to contain also their hybrids. The specimen of 1807 is designated *O. biennis*, but both the flowers have the lobes of their stigma above the anthers, which is a differentiating mark of *O. Lamarckiana*. Moreover, it is the only decisive detail, all other characters of the figures applying equally to both species. If it is allowable to trust to this detail, we should be entitled to conclude that the station of Liverpool contained both forms as early as 1807, even as it is known to do at the present time. In this case, *O. Lamarckiana* must be assumed to have been introduced into England about the time of MICHAUX and LAMARCK, and a common origin for the specimens of their herbaria and the wild stations in England becomes highly probable.

The strain of Carter and Co. has been identified by LINDLEY as *O. Lamarckiana* Ser., and the high authority of this eminent botanist confirms my own determination of the same strain, made by comparing it with the authentic specimen of LAMARCK.[31]

At all events, the adduced facts indicate a very simple history of our species, which has come down to us unchanged, so far as we know, from the original American habitat.[32] There is no reason to suppose that it originated as a garden plant, and none at all to subject it to all the doubts ordinarily brought forward against the purity of descent of horticultural forms in general, simply on the ground that some garden plants are of known hybrid origin. *O. Lamarckiana* has remained unchanged through more than a century, and has kept as true to its type as any good wild species. "It is exceedingly fortunate," says DAVIS (*op. cit.* p. 527), "that the plant which serves as the type of *Oenothera Lamarckiana* Ser. should have come down to us so well preserved that there is scarcely a doubt of its identity." But the identity is with the species as it is still known under that name. Whether the species

[31] DAVIS says (*op. cit.* p. 531) "the identification by LINDLEY of these plants with *O. Lamarckiana* Ser. was undoubtedly incorrect," but he does not give any reason for this assertion.

[32] DAVIS says (*op. cit.* p. 530) "that *Lamarckiana* has come down to us greatly modified, that its parentage is far from pure, that it is in fact of hybrid origin." This assertion, which is not based upon any facts, is clearly contradicted by the preservation in excellent condition of the three specimens of LAMARCK, POURRET, and MICHAUX, not known to DAVIS.

was in the same condition of mutability at the time of its first appearance as it is now, is of course a different question.[33]

Summing up the results of this historical investigation, we may say:

1. *Oenothera Lamarckiana* Ser. is represented by specimens in the herbaria of LAMARCK, POURRET, and MICHAUX (pls. XVII–XIX), and is, so far as this material enables us to judge, at the present time exactly the same plant as it was at that period. It has come down to us, through more than a century, as unaltered and as constant as true species usually do.

2. It has been a component of the flora of the eastern United States, where MICHAUX collected it and whence LAMARCK derived his specimen.

3. At the present time it is a component of the flora of England, and is as well established in that country as is *O. biennis* in different parts of Europe.

4. The strain which is now in cultivation, and which was introduced into the trade about the middle of the last century, was probably derived from some wild English locality, which itself may have come from an introduction into Europe of the seeds collected either by MICHAUX himself or by some other botanist of his period.

AMSTERDAM

EXPLANATION OF PLATES XVII–XIX

Plate XVII

Oenothera grandiflora Lam. (*O. Lamarckiana* Ser.): the authentic specimen in the herbarium of LAMARCK, two-thirds natural size, referred to as *A* in the text; in the left upper corner a bunch of flower buds of my culture of 1913, dried and pressed, is given for comparison, and photographed together with the main specimen.

Plate XVIII

Oenothera grandiflora Lam. (*O. Lamarckiana* Ser.): the specimen in the herbarium of Father POURRET, one-third natural size; on the label is written *Onagra vulgaris grandiflora* Spach.

[33] Über die Dauer der Mutationsperiode bei *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. **23**:382. 1905.

OENOTHERA LAMARCKIANA SER.

HERBARIUM OF LAMARCK

OENOTHERA LAMARCKIANA SER.

HERBARIUM OF FATHER POURRET

OENOTHERA LAMARCKIANA SER.

HERBARIUM OF ANDRÉ MICHAUX

Plate XIX

Oenothera Lamarckiana Ser. in the "Herbier de l'Amérique septentrionale" of ANDRÉ MICHAUX, collected about 1800 in the eastern parts of the United States: *A*, top of spike photographed and reproduced about natural size; *B* and *C*, the whole specimen of MICHAUX, consisting of two parts, reduced about one-half; all three figures photographed for me by Dr. L. BLARINGHEM; in the reproduction the narrow bands of paper used to fix the specimen to its sheet and seen on the photographs have been omitted.

THE SPUR SHOOT OF THE PINES

ROBERT BOYD THOMSON

(WITH PLATES XX–XXIII AND TWO TEXT FIGURES)

Introduction

The deciduous spur shoot, with its limited growth and persistent whorled needle leaves, is the distinguishing vegetative feature of the genus *Pinus*. This structure has been generally regarded as a specialization, the more primitive form of the foliage being indicated by the single spirally arranged leaves which occur on the seedling and in some forms on the adult plant after injury. JEFFREY, however, has raised the question recently of the primitive or specialized character of the fascicled foliage of the pines in his work on the phylogeny of the conifers. He states (**15**, p. 331) that the spur shoot is "a primitive attribute of the coniferous stock" which "has persisted at least in a vestigial form, in connection with the reproductive apparatus, long after it has disappeared, or almost disappeared, in the vegetative axis of the living conifers, with the exception of the very ancient genus *Pinus*." This view is so entirely at variance with so many foliage features, in both the living and fossil forms, that it is difficult to see how JEFFREY could have "cast the balance of evidence" in favor of it. Since, however, he makes much of this spur shoot argument in presenting his case for the ancestral position of the Abietineae among the other conifers, it is desirable to direct attention at least to the most important features of the evidence which is opposed to this view. The writer (**27**) has already stated, in a brief and general way, some of these features, in a paper dealing with the relative antiquity of the Abietineae and the Araucarineae, from the standpoint of their anatomy. New material, however, has recently come to hand which has prompted this more extended treatment of the subject. The literature has also been thoroughly canvassed for information on the spur of the pines.

Of the conifers there are four genera with fascicled leaves: *Cedrus*, *Larix*, *Pseudolarix*, and *Pinus*. In the first three of these

the leaves are numerous, while in *Pinus* they are much more restricted in number, not more than 8 having been recorded up to the present. In the former, too, the number is indefinite and the leaves are spirally arranged, while in *Pinus* the definite cyclic arrangement has been established. A parallel to this is seen in the angiosperm flower, where the lower forms of both monocotyledons and dicotyledons have an indefinite number of floral parts in a low spiral, while in the higher forms the number is definite and the arrangement cyclic. The spur shoot of *Pinus* is deciduous in the second to the twentieth year (ELWES and HENRY **7,** p. 1002), and its leaves fall with it, remaining permanently attached. In the other genera it is the spur shoot which is persistent, and the leaves are cast either annually or in the second to the fifth year. The small and more or less definite number of persistent and whorled needle leaves and the regularly deciduous character of the spur shoot are features which render this structure in the pines very unlike the ordinary branch and also unlike the spurs of the other forms, which differ from an ordinary branch only in their limited primary and secondary growth. The features that will receive attention in this paper are such as indicate the branch character of the spur in *Pinus*.

Number of needles

The number of leaves in the fascicles of the pines, appearing on first sight constant, and being, as ENGLEMANN says (**8**), "the most obvious distinctive character," has been extensively made use of in their classification. He states (**8,** p. 161) that "the sections of 2-leaved, 3-leaved, and 5-leaved pines were the only ones known to the older botanists"; to these were added two other sections "by LINK (*Linnaea*, 1841), one with 2 or 3, the other with 3 or 4 leaves in a sheath." Subsequently forms with single leaves and others with 3–5 leaves in the fascicle had to find a place. Numerous exceptions to this classification have also been recorded. KRONFELD (**16,** p. 68) gives a summary of the variation in many different species observed up to the date of his article. He cites, for example, the occurrence reported by REICHARDT of 3, 4, and 5 needles in *P. silvestris*, which is normally bifoliar, and also of 3, 4, and 6 in

P. Cembra, which has usually 5 to the fascicle. To Dr. G. R. SHAW I am indebted for another reference to the 3 to 5-leaved condition in *P. silvestris* (see KIRCHNER, LOEW, and SHROETER, Lebensg. d. Blütengepfl. Mitteleur. I, 187) and for one to *P. halepensis*, a bifoliar form which may bear 3, 4, or 5 leaves (see MATHIEU, Fl. Forest, ed. 4, 608). In *Gardeners Chronicle* of 1852 (p. 693) an anonymous writer speaks of having raised a variety of *P. austriaca* (normally bifoliar) with 3 leaves. He speaks of these fascicles as being all over the tree, which was about 10 feet high and very dense. The same writer says: "I also find *Pinus Hartwegii* still halting between two opinions between a 3-leaved and a 5-leaved fir. . . . . *Pinus mitis*, *P. variabilis*, *P. muricata*, and others are too well known in their similar tendencies to need remark. My *Pinus insignis* has many a group of 4 leaves, instead of the prescribed 3." In *P. macrophylla* he found fascicles with 6 and 7 needles quite common, even some with 8. SHAW himself (**26,** p. 6), in his description of the pines of Mexico, encountered so much variation in four of the nut pines (*P. cembroides*, *P. monophylla*, *P. edulis*, and *P. Parryana*) that he said: "I find it impossible to separate these specifically, their cones being identical and the number of their leaves inconstant." The leaves in the foregoing instance varied between 1 and 5. He has also recorded (p. 23) a great variation in single species. Of *P. Montezumae* he says: "Trees bearing fascicles of 6, 7, or 8 leaves are quite common, but such excessive numbers are found usually on older trees and in favorable years. On young trees fascicles of 3 and 4 leaves may be found, but in all cases fascicles of 5 predominate." Of *P. ponderosa* the same author states that the leaves are in fascicles of 2–5, but has found fascicles of 6–8 on mature trees. *P. Teocote*, *P. patula*, and *P. Lawsoni* agree in having usually 3 to the fascicle, but the first two have occasionally 4 or 5, while this is more usual in the third. In *P. leiophylla* the conditions are evidently reversed, since SHAW (p. 13) says that the leaves are "in fascicles of 5 or of 3 and 4." SARGENT (**24,** p. 119) states of *P. serotina*: "The leaves are borne in clusters of 3 or occasionally of 4 on vigorous young shoots," while of *P. heterophylla* he says (p. 157): "The leaves are borne in crowded clusters of 2 or 3, the 2-leaved clusters being most common

on young vigorous trees and on fertile branches." In *P. Pinea*, which usually has the leaves in pairs, ELWES and HENRY (7, p. 1119) state that "on well developed vigorous branches, a few of the leaves are sometimes in clusters of threes." Of *P. Torreyana*, whose adult leaves are in fives, they also state (p. 1065) that "on young plants the leaves are frequently in clusters of 3 and 4." BORTHWICK (1) has described a tree of *P. Laricio* 12 years old with 2, 3, and 4 leaves to the fascicle. The quadrifoliar spurs were found only at the top of the tree, which was of very vigorous growth.

The variations in the number of leaves in the pines have been recorded practically as isolated instances and have not been correlated. As they stand, they show that the spur shoot is not so definite and so specialized a structure as has been supposed, but that it is more in the nature of a branch with an indefinite number of foliage leaves. When, however, one looks farther into the variations from the standpoint of the spur being ancestrally a branch, it is evident that the fascicles with supernumerary needles should be found in the more primitive parts of the plant: on the seedling, on the fruiting branch, after wounding, etc. My own investigations have been along these lines, and though they do not completely correlate the cases reported, they go very far toward doing so and afford one important line of evidence of the branch character of the spur shoot.

Fig. 1 is of the upper part of a 3-year-old seedling of *P. Strobus*.[1] Primordial leaves are unusually persistent on this plant, and may be seen among and below the three spurs with the rubber bands around their leaves. Brown scale leaves, however, replace these green seedling leaves around the base of each fascicle just as in the ordinary spur. The middle spur bears 15 leaves, the one to the right 11, and the one to the left 7. Fig. 2 is of a seedling of the same species, one year older. The main axis in this case made a comparatively short growth the last season and bears 6 fascicles. The central one of these has 9 leaves, the one to its right 10, the two below these 7 each, the lowermost to the right 5 large and 2

[1] I cannot determine absolutely that these and the other young forms are *P. Strobus*. It is possible that they are *P. excelsa*, but, since some consider the two species as geographical varieties, the matter is unimportant from the present standpoint.

small, while the lowest to the left has 6 equal-sized needles. This plant was slightly wounded a year ago last spring in connection with some work a student is doing along another line. It was not injured, however, in such a way as has been found in other cases to interfere with the number of needles. Again, several sister plants similarly injured did not show any reaction. It is probably better to consider this case a "sport," just as in the case of the 3-year-old plant, which had not been injured in any way so far as could be determined. In only one instance have I found younger plants than these with extra leaves in their fascicles; one seedling from a dozen or so of *P. flexilis*, which are now in their second year, has a fascicle with 6 needles. It is among the first formed fascicles of the seedling and at the top of the second season's growth.

It is more usual to find the spurs poorly developed when they first appear on the seedling, which is generally in its second year, though in some species they are delayed till the third year, or even later. This feature shows itself especially in species which have normally more than two leaves in the mature condition. For example, HEMPEL and WILHELM (11) refer to *P. Cembra* seedlings two years old as having trifoliar spurs, though the adult plant is normally quinquefoliate. I have also found trifoliar spurs common in *P. Strobus* when fascicles first appear on the seedling. Some spurs here are even bifoliar. In these reduced spurs the needles apparently come right out of the stem, the shoot axis, if any be present, being imbedded in the tissue. These fascicles also are usually devoid or almost devoid of bracts. In *P. silvestris* I found in 35 seedlings two years old only one example of a trifoliar spur. This was on the most vigorous of the plants. In plants a few years older, which had attained to considerable vigor of growth, I could scarcely find one without trifoliar spurs unless it was a weakling. At first the occurrence of these reduced fascicles on the seedling seemed entirely at variance with the view that the spur shoot of the pines is a branch. It is a feature, however, which is shared by other spur shoot-bearing plants. In *Ginkgo*, for example, when the spurs first appear, about the third year, they bear only 1–3 or 4 leaves, and gradually gain in number as they advance in age until

they attain their mature condition. This is also true of the other fascicle-leaved conifers, sometimes only one or two needles developing in the season that the spurs appear. There must thus be some common physiological reason underlying this feature, and no doubt it is the well known lack of vegetative vigor in the seedlings of the conifers generally. During the first few years they are busy establishing a root system and there is little stem growth. Foresters and nurserymen know this early critical stage in the life of the conifers only too well. The growth in these early years could be measured in inches, while in later years it would require feet.

I have examined older forms, 6–15 years old, of a large number of different species belonging to all the different sections of the pines, and have found supernumerary needles quite common, especially on vigorous specimens. On the main axis of one unusually sturdy plant of *P. Strobus*, a plant which had made at least a foot and a half of stem this year, and this stem fully three-quarters of an inch in thickness at its base, there was a spiral sequence of fascicles with 6, 7, 8, 9, and 10 needles. Fig. 4 is of one of these. The supernumerary needles have been surrounded by a rubber band, and it will be seen that they show a spiral gradation in length. This is a feature which to a less degree is sometimes shown by the 5 original needles themselves. It is indicative of the concealed spiral arrangement of the leaves on the spur. This feature, I find, was observed long ago by MEEHAN (**22**), and its significance noted. The series of spurs referred to above occurred on the lower part of the year's growth. It is more usual, however, to find spurs with extra needles near the apex of the season's growth. I have found many instances of this in a mixed plantation of pines about 8 years of age, which consisted of *P. Strobus*, *P. silvestris*, and *P. Banksiana*. Of the white pine there was a large proportion of vigorous specimens with supernumerary needles. The Banksian pine showed few instances, but in the Scotch pines they were very numerous. I should think fully 75 per cent of these had several (3–6 or 7) trifoliar spurs at the apex of the year's growth. These trifoliar spurs could be traced for two to three years previously at the corresponding places on the stem. The occurrence of trifoliar spurs in this species at the branch region was observed by

the anonymous writer in *Gardeners Chronicle* of 1852, to whom reference has already been made. He says of these spurs in *P. silvestris:* "I have gathered 8 or 10 examples round one bud alone," and adds "on the *macrophylla* the examples are very numerous," evidently intending it to be understood that the abnormal fascicles of this species, which he has described with 6–8 needles, were also found in the branch region. I have observed in *P. excelsa* 6–7 needles in the same position, in *P. parviflora* 6, and in *P. virginiana* 3. The first two of these are normally 5-foliate, while the third is 2-foliate. In *P. Jeffreyi*, which is normally 2–3-foliate, I have found on plants of about 8 years of age spurs with 5–6 needles; these were often in a terminal position (see fig. 13, where a branch has originated from such a spur). In all cases it is more usual for the extra numbers to appear on the main axis than on branches, though I have found them on the same plant in both places. I have examined fruiting branches in the case of *P. Strobus* and *P. excelsa* only, and have found several instances of 6-needled fascicles on these.

This normal production of supernumerary leaved fascicles, as it may be considered, is interesting, but of much greater interest is their traumatic production. The past summer, Mr. J. R. FRYER was experimenting with white pine along this line and succeeded in producing on young trees, about 15 feet high, fascicles of 6–8 leaves, in one instance 11. He cut the young growth from vigorous branches and main axes in the latter part of May, and when new growths arose they had in some cases the extra needles in the fascicles. In *P. excelsa*, at about the same height, I found in the middle of July many such cases, where the terminal bud had been injured in the early spring (probably by the pine shoot beetle, *Hylesinus piniperda*) when the fascicles were beginning to develop. The wounding caused numbers of these fascicles to develop extra leaves. Fig. 3 gives a fair illustration of the tufted appearance of these shoots and shows three fascicles with 9–11 needles (see also in fig. 10 the central fascicle). In some instances the number of leaves reached 15. In *P. parviflora* I have found on a young tree fascicles of 6–9 needles. These were near the top of the main axis, which had been slightly wounded lower down. Even in the

single-leaved pine, *P. monophylla*, wounding increases the number of needles to two. Figs. 5 and 9 are from a young tree which was normally monophyllous. Both twigs were injured just as the leaves were starting to develop. On the first, three bifoliar spurs have been produced, and on the second, one. I have not observed an increase in the number of needles by wounding in any other forms, but it is probably of quite general occurrence in the pines.

Before leaving the subject of the number of leaves in the fascicle, a peculiar and probably a specialized condition, which has been reported in several species, must be referred to. In *P. Nelsoni*, SHAW (**26,** p. 8) states that the leaves are "connate in fascicles of 3," and that this condition is found even in the seedling. In *P. Thunbergii* the leaves are in twos, while "in var. monophylla the two leaves in the cluster coalesce" according to ELWES and HENRY (**7,** p. 1143). These authors also refer to two other cases of fusion of the leaves; to CARRIÈRE'S (Conif. p. 398, 1867) description of a variety (*monophylla*) of *P. excelsa:* "each sheath with apparently only one leaf, all of the five leaves being welded together" (p. 1011), and to a monophyllous variety of *P. Strobus* described by TUBEUF in 1897, "a variety with the needles more or less cohering throughout their length, and forming a single needle" (p. 1026). It is necessary to distinguish this spurious monophyllous condition, a result of compounding, from the truly single-leaved condition in the one species, *P. monophylla*, which as MASTERS (**20**) has shown arises from the "arrested development of one of its two original leaves" (p. 126).

Scale and primordial leaves

Below the persistent whorled needle leaves on the spur shoots are spirally arranged scale leaves, which are homologous with the similarly though more laxly arranged scale leaves on the ordinary branches. They are either persistent or deciduous. On seedling stem and branches scale leaves are replaced by the so-called primordial leaves, which are a prominent feature in the pines. These seedling leaves, as COULTER and CHAMBERLAIN (**5,** p. 222) have noted, are of simpler structure than the whorled needles. There

are gradations in structure, however, between the two types of foliage. BORTHWICK (1, p. 153) refers to this feature in his description of the supernumerary needles of *P. Laricio*, to which reference has been made. He says that "the fourth needle, ... although it shows some of the primary leaf characters internally, still, in outward appearance, it resembles the normal acicular leaves, exhibiting in fact a transition stage between the two." I have found very complete series of transitions in form between both scale and primordial leaves, and also between these and the whorled needle leaves. The latter is well indicated in fig. 10, where the upper spur (5-foliate) has proliferated into a branch with primordial leaves. It is possible here to tell the spur leaves from the others only by their low spiral arrangement and by their slightly triangular rather than flat form. In the fascicles illustrated in fig. 3, some of the upper bracts have been modified into green seedling-like leaves. The needles of these fascicles, too, are flatter, shorter, and more like the primordial type. This is especially true of the needles of the fascicles in the younger plant shown in fig. 1. More definite reference to these points will be made in a future paper dealing with the internal structure. The morphological evidence, however, seems sufficiently clear that the spur shoot leaf is only a specialized primordial leaf, just as the scale leaf is also a modification of it.

The transformation to both types of foliage occurs at somewhat different stages in the life of the seedling of various pines. Of *P. monophylla*, SARGENT (**24,** p. 51) says: "primary leaves are the only ones produced during the first five or six years in the life of the plant"; while BRITTON (**3,** p. 14), in referring to this feature in *P. cembroides*, states: "juvenile leaves of this and other nut pines are produced for the first five years or more, often to the exclusion of all others . . . . the new ones shorter as the buds of the fascicled, needle-shaped leaves develop in their axils." MASTERS (**21,** *fig. 1*, p. 586) has figured the persistent primordial leaves of one of these, *P. Parryana*. He previously figured those of *P. Pinea*, a form in which he notes that they were observed long ago by LINNAEUS (MASTERS **19,** p. 258 and *fig. 8*). Of the last mentioned species, ELWES and HENRY (**7,** p. 1120) state that "the primary leaves are produced . . . . for several years, in mixture,

after the first season, with the adult geminate leaves." LLOYD'S (18) attention was attracted to the great persistence of the primary leaves of a young plant growing in the New York Botanic Gardens. In *P. canariensis* the leaves persist even longer than in *P. Pinea.* In *P. rigida* they are also very persistent. In the seedlings of most forms, however, the primordial leaves do not last beyond the second or third year.

Primordial leaves are not, however, restricted to the seedling. According to MASTERS (**19,** p. 258), "they occur frequently on the lower part of the shoots of the year, as in *Pinus sabiniana, Pinea, silvestris* (sometimes), and other species"; also "in some cases, on the branches or stalks immediately supporting the cones, as in *Pinus excelsa,* etc." In *P. monophylla,* ELWES and HENRY (**7,** p. 1056) have noted that "in cultivation adventitious shoots bearing flattened primordial leaves are occasionally produced on the lower branches." SHAW (**25,** p. 206), in speaking of the "summer" growth of certain southern pines of the United States, says: "this growth, in the summer, differs from the spring growth not only in its less development, but also in its *green* bracts, which, not being required for the protection of the winter bud, assume more or less completely the size, color, and character of the primary leaf." SARGENT (**24,** p. 4) states that: "*Pinus rigida* and *Pinus echinata* are the species of the United States which generally bear primary leaves on branches, or produce freely shoots from the stumps of cut trees. These shoots, which are clothed with primary leaves, grow vigorously for a few years, and then usually perish." ENGELMANN (**8,** p. 163) speaks of this feature in *P. inops, P. rigida,* and *P. canariensis.* In the last mentioned, it is very prominent in some instances. Miss COOLEY (**4**) refers to and figures a tree at Naples which was practically clothed with shoots bearing primary leaves. A young specimen of the same species in the New York Botanic Garden shows many reversions to primary foliage. Whether in these instances all the reversions may not be the result of injury is uncertain. Wounding does give a response in the case of the production of resin canals for several years after the injury, especially prominent being the response in young twigs which are formed subsequently to the wound, and it is probable

that the extent of the primary leaves due to wounding is much greater than at first appears.

The possibility of reviving the primary type of foliage by wounding must be fairly common in the pines, for in addition to the ones that have been mentioned, MASTERS (**19,** p. 258) refers to their production after injury in *P. edulis*, *P. Parryana*, and *P. Khasyana*. The past summer I have observed it in ten or more species: *P. canariensis*, *excelsa*, *halepensis*, *Jeffreyi*, *Laricio* and var. *austriaca*, *monophylla*, *Pinaster*, *Pinea*, *ponderosa*, *Thunbergii*, and *tuberculata*. LLOYD, moreover, has produced the primary type of foliage experimentally in *P. ponderosa*. He states (**18,** p. 101; see also original article, **17**) that "shoots, which normally would bear only thin, brown, papery scales, namely the shoots which bear the male or pollen-bearing cones, may be made to produce true primordial leaves by the mere pruning away of the upper part of the shoot early in the spring." HOCHSTETTER (**12**) has gone farther, and has succeeded in fixing the juvenile foliage in *P. Pinea* and *P. canariensis* by cuttings, having accomplished in this specialized genus of the Abietineae what is common practice in the Cupressineae. He states (p. 367): "Stecklinge von *Pinus canariensis* und *Pinea*-Sämlingen, im zweiten oder dritten Jahre abgenommen, wachsen leicht an, verharren in der Primordial-form und bilden bläulich-grüne Büsche mit spiralig einzeln gestellten Nadeln von unvergleicher Schönheit." Unfortunately, these "incomparably beautiful" shrubs are but short-lived. Had they succeeded better and become disseminated through horticulture, they would have afforded a convenient and valuable demonstration of the primary type of foliage of the pines.

In some seedlings of *P. Strobus* in the third year I have found a reversion to primordial leaves where no wounding could be detected. The leaves, for example, shown on the branch above and to the left of fig. 11, though broader and more closely set than usual, are primordial leaves produced after the plant had formed spur shoots. Such leafy branches may even originate from a spur shoot, three stages of differentiation being shown in the figure. To this point, however, reference will be made again. MASTERS has observed (**19,** p. 258) in *P. rigida* and *P. silvestris* that if the main axis is

injured slightly above the cotyledons the branches which are produced in response to the wound bear only primordial leaves. He considers that this doubtless occurs in many others. From these facts and the stump sprouting, which has been referred to above, it seems probable that in all cases the epicotyledonary region, in the young plant especially, reverts more readily to the primordial type of foliage than the upper parts of the stem, and that what seems more or less normal here and, in some cases below the cones, can be made to occur in other parts by wounding. PHILLIPS (**23**) has recorded some observations bearing on this point. Speaking of the sprouting of *P. chihuahuana* after injury by cattle and fire, he says (p. 385): "typical sprouting . . . . is confined to trees under 5 cm. in diameter (measured at breast height), which send up most of the shoots from the root collar or the first 30 cm. above ground." This power of stump sprouting, as it is called, also decreases with the size of the stump: "Not a single case was found where the stumps of trees smaller than 7.5 cm. in diameter had failed to produce thrifty sprouts, and fully 30–50 per cent of the stumps of trees up to 22.5 cm. in diameter had produced very thrifty sprouts, most of the fail stumps occurring between the 15 and 22.5 cm. classes" (pp. 386–387).

Proliferation of the spur shoot

The spur shoots, like ordinary branches, arise either in the axils of primordial leaves or, when these have been replaced on the stem by scale leaves, in the axils of the latter. Unlike the branch, however, the spur shoot of the pines increases neither in length nor in diameter after the first few weeks, though it may remain on the tree with leaves green and apparently functional for many years. Thus, primary meristem and cambium are normally inactive through by far the greater part of the life of the spur shoot. This limited growth of the spur shoot and the production by it of only one set of assimilatory leaves which are persistent, not even detached on the fall of the shoot, are the distinguishing features of the spur of the pines. In the other fascicle-leaved conifers, a new set of leaves is added to the spur shoot each year for many years (cf. *Ginkgo*). There is thus a slight annual increase in length, the

spur shoot elongating, much after the fashion of the cycad stem, enough to accommodate each new set of leaves. The "spurs" in these forms may attain a length of an inch or more on the older branches; they gradually become shorter toward the younger parts, and on the season's growth primordial leaves alone may be present. These persist in *Cedrus* for two to three years. The occurrence of these primordial leaves on the season's growth are a contrast to the condition in the pines, where only scale leaves are normally produced. In *Cedrus* the fascicled leaves themselves persist for two to five years, while in *Larix* and *Pseudolarix* they are shed annually. In the pines they are indefinitely persistent, falling with the spur. Undoubtedly the persistent habit is the ancestral one for the conifers, having been overcome in two ways, by the deciduous leaf and by the deciduous branch, as the writer has tried to show in a recent article on the Araucarineae (**27**).

The spur shoots of the three genera referred to, in addition to having the power of making a short yearly increase in length, have also the power to a marked degree of forming ordinary branches as occasion arises. The main axis may originate from a spur shoot, usually the terminal one, but if this is injured, any of the proximals may assume its function. Lateral branches also arise normally from spur shoots, especially from the younger ones. When, however, a branch is injured, they may arise from old spurs. The spur shoot of these three genera retain to a marked degree the power of branch formation. It remains, however, to a large extent latent unless called into activity by the needs of the plant. This dual power of the spur shoot either to produce a branch or to continue the growth of the fascicle as such is an indication of the genetic relationship of the branch and spur shoot. One would scarcely expect such a feature in the pines, where ordinarily in the mature condition not even a rudiment of a bud can be discerned amid the closely set needles of the fascicle, and where, too, only the one set of leaves is produced and the axis neither increases in length nor in diameter after it has formed these.

On the contrary, however, proliferating spur shoots similar to those in the other genera are of very general occurrence in the pines. They do not occur so abundantly nor so normally as in

the other genera, and have escaped observation to a great extent. Several instances, however, are on record. ENGELMANN (**8,** p. 167) says: "in exceptional cases and as a monstrosity the leaf bundles may become proliferous, the branchlet which bears the secondary leaves elongating and forming a regular branch." MASTERS (**19,** *fig. 9* and p. 267) figures a pine, with two needles, in which the fascicle is "prolonged into a shoot with primary leaves and leaf buds." Neither ENGELMANN nor MASTERS mentions the species concerned, nor in either case do they indicate the conditions which have induced the proliferation. DICKSON (**6**) and MEEHAN (**22**) both observed that in *P. silvestris* proliferation occurred as a result of injury. DICKSON's specimen was a twig, the extremity of which had been destroyed. He says (p. 260): "the development of these buds is stronger the nearer their position to the seat of injury." The lower ones are merely closed buds, but the upper ones "develop well marked foliage leaves, and, in the very strongest ones, these foliage leaves have secondary bifoliar spurs developed in their axils." In the development of foliage leaves spirally arranged "on the prolonged axis of the stimulated spur," DICKSON notes "a reversion to the early or unspecialized condition." MEEHAN'S (**22,** p. 82) specimens were Scotch pine that had been "headed back." Previous to his observation of these proliferating spur shoots, he had been so influenced by VON MOHL'S work on *Sciadopitys* that he was inclined to consider the fascicled needles of the pine as cladodes. He now subscribed to the current view and recognized that the whorled needles are leaves and that the fascicle is an "arrested branch, having a dormant bud at the apex." BORTHWICK (**2**) has observed numerous instances in a plantation of *P. Laricio* and *P. silvestris*, and has studied the conditions under which they develop, as well as the character of the resulting branch. He considers that "the interfoliar bud develops only under the stimulus of an increased supply of nutriment," and instances several kinds of wounding which result in a greater advention of food material. In this he agrees with HARTIG'S explanation of the development of dormant buds. He considers that the branch produced from the development of the interfoliar bud may bear either primary or fascicled leaves, "the results

varying with the conditions under which the buds are induced to develop and the general health of the tree at the time" (p. 157); the greater the supply of nutriment, the more likely it is that the proliferating branch will produce spurs, the primordial foliage occurring on those with the smaller supply. BORTHWICK has even referred to an economic aspect of the proliferation of the spur. He considers that the appearance of scraggy pine trees could be improved by a judicious disbudding process and a stimulation of the spurs to form branches.

It is in the seedling and in the young vigorous plant that I have observed most instances of proliferation, the occurrence of proliferating spurs following very closely that of supernumerary needles. This applies also to their common production in the mature tree by wounding.

The upper part of a seedling of *P. Strobus* at the beginning of its third year is shown in fig. 6. A branch with young spur shoots (this season's growth) has been developed from the interfoliar buds of four spur shoots of last season's growth. The central one of these will probably form the future stem. The big branch to the lower right of the photograph, whose leaves have been tied together, arose a year previously also from a spur. Above it is a normal fascicle which has not proliferated. The main axis below the leafy part had its end destroyed, as its dead stump shown against the small white slip indicates. Its lower branches, which were uninjured, bore only normal spur shoots, and it is possible that wounding has had something to do with the proliferation of the upper spurs. I found, however, in what so far as could be determined was an uninjured sister plant, one case of proliferation. In another, also apparently uninjured, I found a reversion to primordial leaves, which has been previously mentioned; though the large branch shown to the upper left of fig. 11 did not arise from a spur shoot, at least, if it did, no trace of the fascicle of leaves remains, yet to the right of the terminal bud there is a spur shoot with a small set of primordial leaves, while to the lower left of the figure two other fascicles are shown with smaller series, so small that they are practically green buds. This photograph was taken while the plant was in its winter condition. Two of these fascicles have since

developed into branches with both primary and secondary leaves. I have found similar proliferations on seedlings from the same plot, but could detect no injury except in one instance. The seedlings were all grown and carefully tended in the University Garden. Moreover, in both seedlings shown in pl. XX there are well developed buds in all the spurs except two (the lowermost of fig. 2), some of which will in the ordinary course of events grow next year into branches. In fact, the main axis will come from one of these in both instances, as it has done in the plant shown in fig. 6. One is so much larger than the others in each case that there seems no doubt of its power to produce the main axis.

On examination of older seedlings of other species, I found that branches had quite frequently arisen from spur shoots in certain plants, again not connected with any apparent injury. Among about 20 young plants of *P. virginiana* (?), several had proliferating spurs, and were vigorous specimens about 6 years of age. In fig. 7 the two leaves (marked with a single line each) inclose the terminal axis with a rosette of lateral buds at its apex. These two leaves have very broad bases. Below and to the right is a lateral bud coming from a spur shoot with three leaves (marked with 2 lines each). Two of these are also broad, but the third is much narrower. It is to be noted that this species has its leaves normally in twos. Below this spur shoot is one with two broad leaves (marked with 3 lines each) and a much smaller bud, below which again are spur shoots with smaller and smaller buds, until at the base of the shoot figured no trace of a bud could be discerned, even with a lens. During previous years certain of the branches also arose from spur shoots on this plant. In vigorous specimens of *P. Strobus*, about 8 years of age, chiefly from the plantation referred to before, but in the wild also, I have found that usually subsidiary branches occur below the normal whorl. These are smaller and in almost every instance derived from the proliferation of a spur. One of them at the end of its first season's growth is shown in fig. 8. It has normal secondary needles and is only one of several at the same node. Around the apex of the stem below the branch node, a number of this year's spurs have small buds which are ready to develop next year into similar branches. In a few specimens of

P. parviflora, I found numerous examples of similar proliferations. A large proportion of these branches formed in this way perish later, but some persist and develop normally. Sometimes in these species the spurs which will proliferate have an extra needle. In *P. silvestris* this is normally the case. In vigorous specimens the lower branches on the swollen branch node of this form come regularly from trifoliar spurs. Many of these branches persist, and I have also observed that sometimes the main axis comes from a spur, no special terminal bud being formed. This condition, however, is rare in the Scotch pine at the stage (about 8 years old) examined. In *P. Banksiana* in the same plantation proliferation was not so common as in the other two species,[2] but occurred under the same conditions.

FIG. 1.—*Pinus ponderosa* var. *Jeffreyi* (one-half nat. size): the main axis and a branch developed from spur shoots in a plant about 6 years old.

In some young plants of *P. ponderosa* var. *Jeffreyi*, I have found proliferation very common. These plants were about 6 years of age, and did not exhibit any special vigor of growth. Text fig. 1 shows the main axis and a branch, with some of the needles of the spur from which they arose attached to their basal regions. Others of these needles have become detached. In these plants there are normally only 3 needles to a spur. In the axial stem-producing spur shown above, 4 needles are still attached to the base, and the broken stumps of 2 others can be clearly seen. The branch spur has but 2 needles intact, but the stumps of 2 or 3

[2] Mr. C. H. MORSE, while on a visit to the government nurseries in Norfolk County, was good enough to examine Scotch, Banksian, and white pine, and reports that "proliferation is very abundant, especially in vigorous plants of *P. silvestris* 6 to 8 years of age, and fairly common in *P. Banksiana* and *P. Strobus* of about the same age."

others can be made out on the specimen, though not in the photograph. When one examines the insertions of these needles, they are seen to continue the spiral series of the scales above them, which are more developed than usual and more than usually persistent.[3] The spiral is a very low one in the case of the 3 lowest (the normal) needles, but comes out clearly in the supernumerary needles. In these, too, there is a very evident gradation in length, the leaves getting shorter the higher they stand on the axis. In fig. 13, for example, the 3 large (slightly spirally twisted) needles, though each is inserted at the base of a spiral series, are all about on a level. The other 3, however, are inserted at quite distinct levels. The shortest is the highest, the variation in their length being an inverse index of their position on the stem. This is true of the supernumerary needles of *P. Strobus*, shown in fig. 4, though their relative postions are not indicated. It is very noticeable in *P. Jeffreyi* that it is the spur with the supernumerary needles that proliferates most freely, though sometimes normal fascicles do also.

In general, the needles of fascicles which have proliferated are irregularly deciduous, remaining attached for a good while usually, and often becoming brown and weathered. One can often detect spurs that have proliferated by these needles. I have seen these needles persisting in the Scotch pine when they had become separated as much as three-quarters of an inch by the expanding base of the branch. This forces on one's attention the thoroughness with which the secondary as well as the primary meristems of these fascicles have been revived, the pines in this respect showing complete agreement with the well known condition in the other genera of fascicle-leaved conifers, where, however, normal proliferation is much more abundant. It is significant that normal proliferation in the pines occurs in the branch region of the stem and from fascicles with extra needles or in association with such fascicles.

Only in the seedling have I ever observed that the branch arising normally from a spur bears primary leaves. After injury, however, this is of quite frequent occurrence, especially on young plants. The branch coming from the spur in fig. 10 of *P. excelsa*

[3] Though I did not see these in the young condition, I should infer that they were then green.

has very long green bracts near its base and shows the gradation between fascicled and primordial leaves, to which reference has already been made. I have also found green bracts on the branches from spurs of *P. excelsa*, *P. halepensis*, *P. Jeffreyi*, *P. Laricio*, *P. Pinaster*, *P. Pinea*, *P. Thunbergii*, *P. tuberculata*. These are especially large in the *P. Thunbergii*. I have also observed ordinary proliferation due to wounding in *P. resinosa*, *P. silvestris*, and *P. Strobus*. In most cases these were young trees about 10–15 feet high. In the case of *P. Laricio*, *P. Laricio* var. *austriaca*, *P. resinosa*, *P. silvestris*, and *P. Strobus*, the trees were mature.

In fig. 12 two proliferating bifoliar spurs from an adult Scotch pine are shown. It will be noticed that they are the two uppermost spurs (cf. DICKSON'S observations cited above), and that the injury was done to the apex of the shoot of the year when the little spurs were just developing, not more than one-half an inch in length (see the one attached to the dead apex of the main axis). This is a feature which is usual in other species as well. When the injury is done to the terminal part of the young developing shoot, I have always found a proliferation of the spurs below the injured part. This never extends to the needles of a previous year, nor have I observed that a spur shoot bud can ever be revived after it has remained dormant over the winter, unless some preliminary growth took place the first year. This feature, however, is being investigated by Mr. FRYER, who is carrying on a series of wound experiments on the pines in order to determine at what season, on what year's growth, etc., the best proliferation can be obtained by wounding. He has not found that in the mature tree the spurs of *P. Strobus*, when a year old, can be induced to develop, though those of the season's growth do so very abundantly if wounded early. His test cases were with twigs which had proliferated last year. He tried to arouse the dormant buds of neighboring fascicles by cutting away all but the lowest and smallest of the proliferated spurs. Only those that had grown the year before showed any signs of doing so this year. It seems probable, then, in this species that when the bud of the spur has lain dormant over the winter it cannot be revived. The leaves in this form are shed in the second year, and whether or not those with more persistent spurs can be

made to proliferate after they have undergone one or several winter resting periods has yet to be determined. According to my own observations, it is much easier to induce a young vigorous tree to proliferate than an old slow-growing one, early wounding of the tip of the bud of the season being most effective.

General statement and conclusions

The lack of definiteness in the number of leaves in a fascicle, and the occurrence of supernumerary needles in the recognized primitive region and after wounding, are evidence of the branch character of the spur of the pines. The normal occurrence of single spirally arranged leaves in the seedling, their appearance at times on the cone-bearing branch, their traumatic revival in many instances in the adult, and the transitions which have been found between them and both scale and fascicled leaf, practically demonstrate that ancestrally the leaves of the pines were spirally arranged on ordinary branches, and that the spur is derived from this condition. Its normal proliferation in the seedling and young plant into an ordinary branch with both primordial and fascicled leaves and the traumatic revival of this condition in the mature tree place this conclusion beyond reasonable doubt. In all these features the pines differ from the other spur shoot-bearing conifers only in degree, in conformity with their more specialized condition. If in the one case the spur is a branch, it certainly is in the other. The pine spur shoot, moreover, is wholly vegetative, while in the other forms it is less specialized and combines both the vegetative and the reproductive functions, as is the case in *Ginkgo*, the most primitive of our living spur shoot-bearing forms.

When one comes to compare the conditions in the living pines with their fossil progenitors, several important points develop, which bear out the branch character of the spur. FONTAINE (**9**) has described several species of pines from the Potomac or Younger Mesozoic, having had to modify HEER'S genus *Leptostrobus* slightly for their reception. These remarkable pines had needle-shaped leaves "scattered on the larger or principal stems and grouped in bundles on the ends of short twigs" (p. 227). Some of the fascicles from FONTAINE'S work are reproduced in text fig. 2. They bore

more needles than any pine of today does normally, but in this feature and in the fact that they have considerable axis supporting them they remind one of such fascicles as those of *P. excelsa* (fig. 3), which were produced after bud injury. The fascicles of the fossil form, too, are described as terminal as well as lateral, and must have grown out into branches and the main axis, just as has sometimes been observed in the living pines. The lack of differentiation, too, between the primordial and fascicled leaves and the persistence of the former on old branches afford a full explanation of the transitions which have been found between these leaves in the living pines and also of the occasional "revival" of primordial leaves on old trees. In fact, no better "generalized type" could be desired for the ancestors of the pines than FONTAINE has described. Two other fossil forms have also significant features. In *Prepinus* of the Cretaceous, whose discovery we owe to JEFFREY (14), the spur shoot has 20 or more leaves. These leaves are not cyclic, but are spirally arranged, and are stated to be subject to considerable variation in number. This spur shoot is nothing more nor less than a small branch with closely set leaves, very similar to the condition described in the seedling of certain pines and in the adult after injury. This spur is apparently deciduous, however, but JEFFREY (15) has described one from the Triassic (*Woodworthia*) with very persistent spur shoot, remaining 50 years or more attached.[3]

FIG. 2.—*Leptostrobus longifolius:* from FONTAINE (9, *pl.* 102); reduced one-half.

[3] It is recognized that JEFFREY considered this form a pine specializing in the direction of the araucarians and not as an ancestor of the pines and their allies. Since, however, it has been recently shown that no authentic abietinean forms have been described in the strata prior to it (see GOTHAN 10 and THOMSON AND ALLIN 28), this form must stand as the ancestor of the pines and their allies until some other fascicle-leaved conifer displaces it.

This is certainly a very branchlike feature, and it is noteworthy that it occurs in the most ancient spur shoot-bearing conifer on record.

The paleontological evidence afforded by the fossil pines supplements that from the living forms, and makes the case for the specialized character of the spur shoot of the pines practically complete. The spur then, as it stands today, is only a specialized branch which is of limited (primary and secondary) growth and bears a limited number of specialized and cyclically arranged leaves; whereas its progenitor, judging from the adult living and fossil forms and from the seedling and traumatic phenomena above described, was an ordinary branch. If such is the case, the genus *Pinus* is a specialized one in respect to its fascicled foliage, and the spur shoot of the pines cannot be the indication of primitiveness which JEFFREY'S statement, quoted in the introductory paragraph, would lead us to expect. According to it, the fascicled condition of the leaves is "a primitive attribute of the coniferous stock." This condition, he considers, has been retained in the cone of all, but "in the vegetative parts of only the very ancient genus *Pinus*." It is not apparent why *Pinus* is singled out for this distinction and not some of the other spur shoot-bearing conifers, or *Sciadopitys* whose cladode is recognized by all adherents to the "brachyblast" theory of the cone structure as the closest approximation in the vegetative parts to the condition in the cone scale. Moreover, JEFFREY, in stating the arguments in support of his view, puts much emphasis on the homology of the cone structure of the living and cordaitean forms (**13**, p. 23). The latter, he, in common with many other anatomists, regards as the ancestors of the conifers. If JEFFREY'S inference that fascicled leaves are ancestral for the conifers because of the brachyblast structure of the cone, be applied to the cordaitean forms the logical conclusion is that they must have had their leaves in fascicles. Of this there is not a vestige of evidence. All that the "brachyblast" theory postulates in this regard is the power of branching, a feature which is common to both Cordaitales and Coniferales. If, moreover, the spur is "a primitive attribute of the coniferous stock," we should expect some indication of this in the primitive regions of the non-fascicled conifers; in their seedling, on their fruiting branches, as a result of

wounding, etc. There is no more evidence of this than of fascicled foliage in the cordaitean forms.

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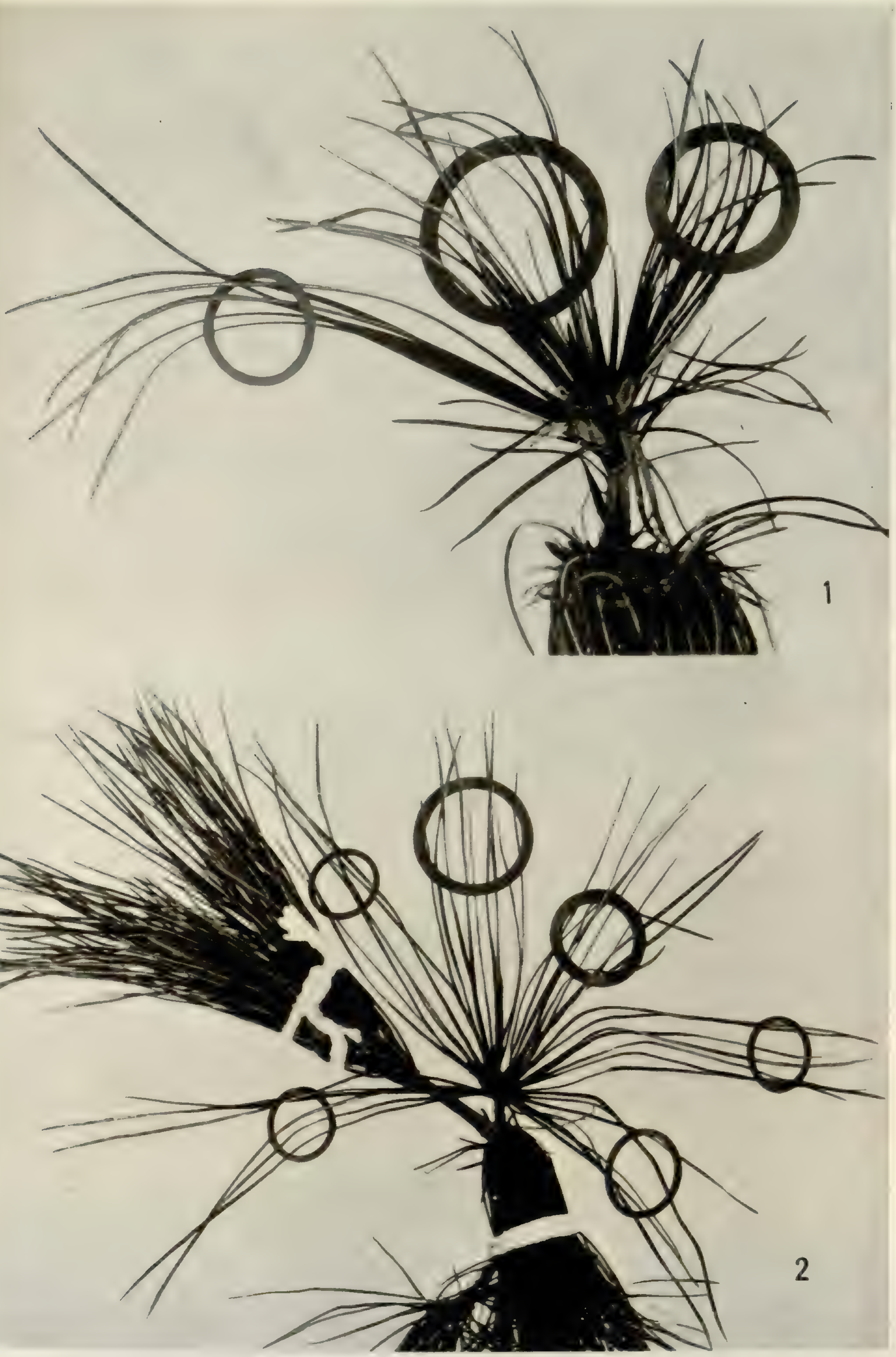
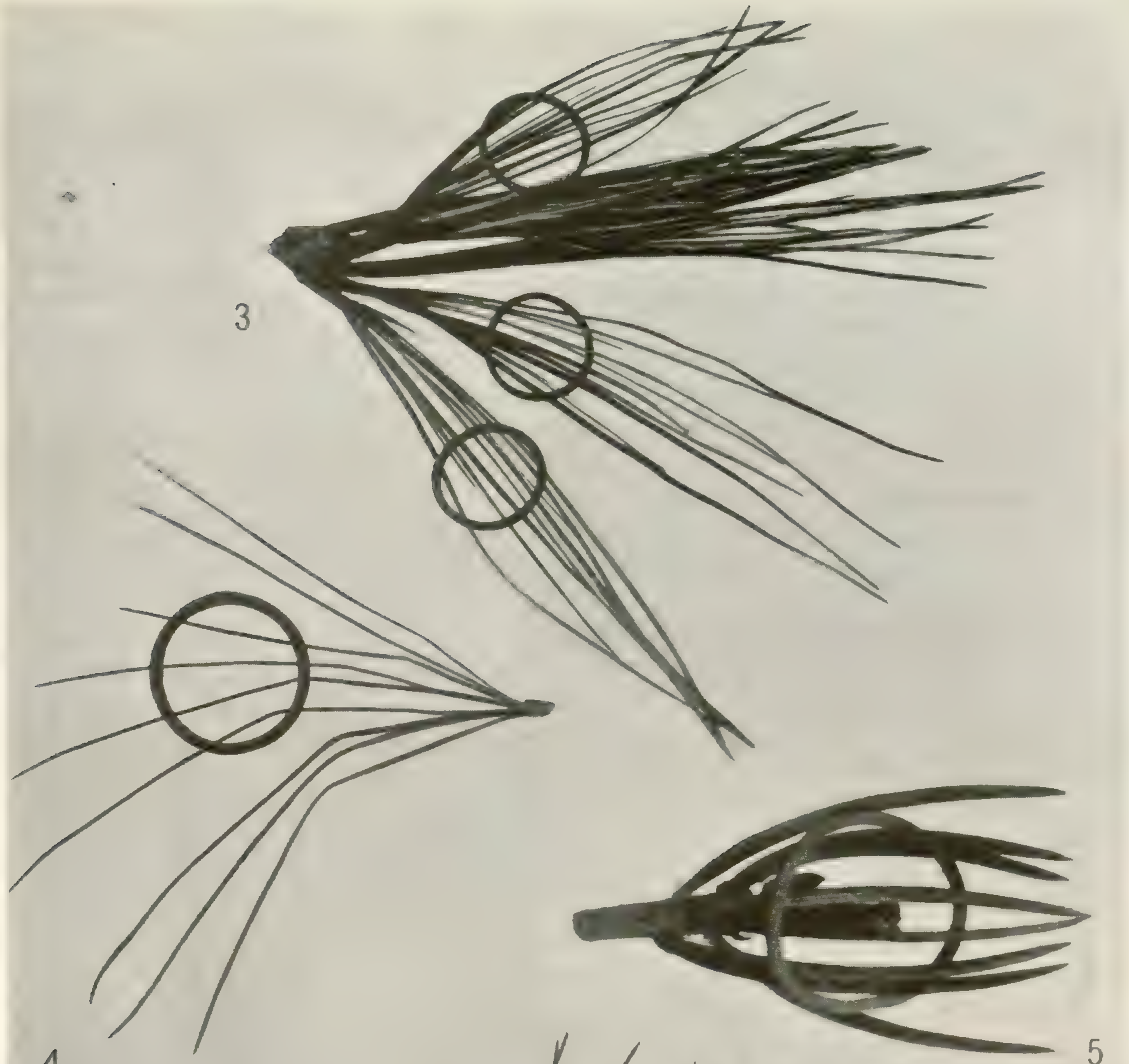


THOMSON on the SPUR SHOOT



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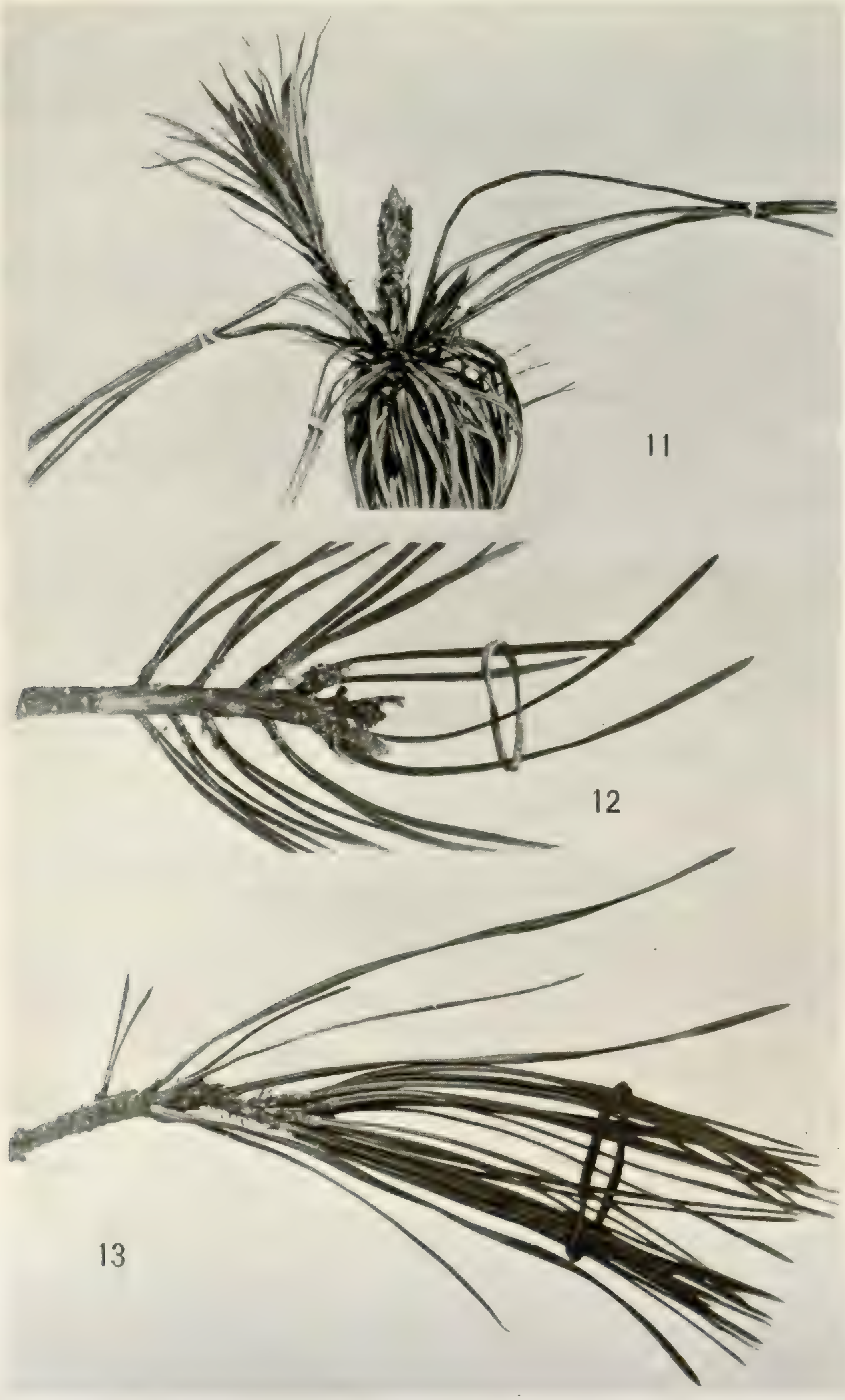

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EXPLANATION OF PLATES XX–XXIII.

FIG. 1.—*Pinus Strobus*, 3 years old.
FIG. 2.—*Pinus Strobus*, 4 years old.
FIG. 3.—*Pinus excelsa*.
FIG. 4.—*Pinus Strobus*.
FIG. 5.—*Pinus monophylla*.
FIG. 6.—*Pinus Strobus*, 3 years old.
FIG. 7.—*Pinus virginiana*.
FIG. 8.—*Pinus Strobus*.
FIG. 9.—*Pinus monophylla*.
FIG. 10.—*Pinus excelsa*.
FIG. 11.—*Pinus Strobus*, 3 years old.
FIG. 12.—*Pinus silvestris*.
FIG. 13.—*Pinus Jeffreyi*.

A PHYSIOLOGICAL STUDY OF THE GERMINATION OF AVENA FATUA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 185

W. M. Atwood

(WITH THIRTEEN FIGURES)

I. Introductory

The apparent inability of many seeds to germinate for some time subsequent to harvest has been the object of study both in Europe and in America. The vitalistic viewpoint, which early ascribed such phenomena to the inherent properties of protoplasm, obscured the whole situation. Such germinative delays were found capable of modification by external influences which were said to exert a stimulus on the seed. The later physiological studies have attempted to uncover the nature of the modifications in function which the so-called stimuli bring about.

Many seeds with low germinative power after harvest acquire increased viability in succeeding weeks or months, during a period which has been termed the after-ripening or germ-ripening period. The changes taking place during this period have been found, in general, due to alterations in the structures inclosing the seed, or to modifications in the inclosed members themselves. The term after-ripening has been limited to changes of the latter type (22), but in this paper will be used to cover all changes in the seed subsequent to harvest, as a result of which greater germination percentages may be obtained.

The most extensive investigations of germination characters among the Gramineae have probably centered on barley, an early and prompt germination of which is much sought in the malting industries. In agriculture much trouble is occasioned in the small grain regions by the persistence of the wild oat, *Avena fatua* L., and by the difficulty with which it is eliminated. As a cattle food it is undesirable because of the long and twisted awns, and also because of its lightness, averaging about 12–18 pounds per bushel. Laboratory and field tests have shown the seed to germinate very

poorly and unevenly after harvest, and later to increase in viability during succeeding weeks. In the work here described, an attempt has been made to determine the factor or factors restricting the germination of the wild oat, and to investigate the nature of the after-ripening changes taking place in the seed subsequent to harvesting.

II. Historical

1. Germination studies of the Gramineae.—It is supposed that the common cultivated oat, *Avena sativa*, has been derived from *A. fatua*. Trabut (**64**) holds that *A. sterilis* and *A. fatua* have given rise to two series of cultivated oats: *A. sterilis* to those of the Mediterranean region, and *A. fatua* to those of Central Europe. Instances are reported for *A. sativa* of what are thought to be reversions toward the parent stock (**52, 67**). The wild oat is specially abundant in the Pacific Coast region, where four varieties have been reported (**20**): *A. fatua*, the true wild oat; *A. fatua glabrescens*, the bastard oat; *A. barbata*, the slender oat; and *A. sterilis*, the fly oat. The wild oat has been found as far east as Illinois, but is not troublesome east of the Mississippi River. It is especially abundant in the northern small grain belt. Recently Criddle (**16**) has been investigating the false wild oats of Canada, which he believes represent some form of deviation from type which affects the seed coat only and leaves the seed unaltered. By the word "type" reference is made to the normal tame oats from which the false wild oats are supposed to have been derived. Yet he finds one of the most conclusive evidences, as to its distinction from *A. fatua* proper, in the greater ease with which the false wild oat may be germinated. These false wild oats resemble certain tame varieties, and may be due to crossing with *A. fatua*, although much uncertainty has been expressed as to the common occurrence of cross-pollination between *A. fatua* and *A. sativa* (**67**). In 1900 Kinzel (**42**) called attention to the progressive rise in germination percentage of *A. sativa* in the months subsequent to harvest. Atterberg (**3**), in his studies of the after-ripening of grain at the Swedish station, finds he can notably raise the after-ripening rate by drying the seed at higher temperatures. Wounding has also

hastened the germination of barley. He believes the temperature at which small grain will germinate is a measure of the degree of after-ripening. Thus, if seed will not grow at 13–15° C., preliminary drying is necessary; while if the seeds are capable of growing at 30° the process of after-ripening is complete. He believes the after-ripening is but the completion of the ripening processes in seeds which have been harvested so late that normal ripening has not occurred. ZADE (67) notes the delay of germination of freshly harvested *A. fatua*, and that it may be influenced by various external factors, but does not attempt to explain the effects of these various factors. He finds the delay is much shorter in seed kept in dry air of the laboratory than in that lying in the field, due as he believes to the difference in temperature. JANSON (38) notes two degrees of maturity in barley and oats, which he calls "yellow-ripeness" and "dead-ripeness." The ripening of oats up to the yellow stage results in a larger increase in the protein stuffs. After yellow-ripeness one-third of the total increment occurs, and is only nitrogen-free stuffs. He thus believes too early cutting results in considerable carbohydrate loss to oats, for the effect of after-ripening extends only to the early stages of normal ripening, which concerns proteins chiefly. KIESSLING (41) recognizes the beneficial effects of drying upon germination, but believes that germ-ripening or after-ripening is a process in general independent of seed-moisture content. He reports an elaborate series of tests under various conditions modifying germination, and concludes as regards oats that germ-ripening is a characteristic varying with the strain tried. He discredits explanations of after-ripening or germinative variations which are based on seed coat exclusions of water or gases, or on enzymatic alterations as so far set forth; yet believes that in some way enzymes are associated with germ-ripening.

2. GENERAL GERMINATION STUDIES.—No attempt will be made to cite all the recent literature on the general field of germination. However, a few instances may be cited to show that such problems have been approached from at least three different angles: (1) studies of germination factors external to the embryo; (2) factors associated with chemical or physical alterations of members within

the seed coat; (3) enzyme studies. In the studies of factors external to the embryo, CROCKER (**17, 18**) found the seed coat obstructs in various cases the entry of oxygen or water. A. J. BROWN in a study of *Hordeum* (**9, 10**) found that non-living structures inclosing the embryo have the power of excluding various salts and acids in solution. This work was extended by SCHRÖDER (**59**) for wheat; while REICHARD (**57**) would explain this peculiarity for barley by saying that there is a causal relation between the solubility of the tannins in the seed coat by various agents and the ability of these agents to penetrate the seed. Wounding and its effect on germination has been noted by various investigators (**12, 17, 30, 31, 39, 41, 65**). The germination rate of *A. fatua* has been increased by alternations of moisture and dryness (**51**). Other factors concerned with germination of seeds as so far studied are drying (**3, 41, 67**), freezing and thawing (**23, 53, 54**), effect of burying in soil (**4, 21**), subjection to various gases during storage and germination (**41**), effects of light (**25–28, 35, 36, 43–47**), of treatment in hot water (**5, 41**), and in dilute acids (**22, 24**).

Chemical and physical studies have been made of alterations in the endosperm or embryo of various seeds. H. T. BROWN (**13**) believes that in after-ripening of barley cytase, acting on the middle lamellae of the cell walls, changes the earlier glazed condition of the endosperm to a mealy structure, following which better germination is possible. JOHANNSEN (**39**) correlates after-ripening with fluctuations in the sugars and amide nitrogen stuffs, while ZALESKI (**68**) has followed the ripening process of peas and found protein increases at the expense of amino acids, amides, and organic bases. KIESSLING cites work (**41**) emphasizing the relation between the protein content in grasses and the speed of germination.

The third type of germination studies, dealing with enzymatic relations, includes a large number of researches. BROWN and MORRIS (**12**) in their study of *Hordeum* believe that the development of amylo-hydrolytic enzymes is located in the epithelial cells of the scutellum, and think the zymogens of the resting seed on germination are activated by the development of acidity. In this view as to the function of acidity in relation to enzymes, they follow the ideas of GREEN (**29**). In 1892 HOTTER (**37**) expressed

the view that the diastatic content of wheat increased in the resting seed, coming to its full possibilities at the time of germination. This after-ripening could be hastened by warming and air-drying of the seeds. DETMER (19) showed that oxygen entry is necessary for the formation of diastase. LEHMANN and OTTENWÄLDER (49) would explain the forcing of germination by various factors as due to hydrolysis of the proteins. These views are based on experiments in which temperature variations were employed, nutrient solutions were used, and also splitting products of the proteins as asparagin. ABDERHALDEN and DAMMHAHN (1) believe that germinated and ungerminated seeds vary in the presence or absence of peptolytic ferments, while APPLEMAN (2) for the potato and Miss ECKERSON (22) for *Crataegus* trace enzymatic alterations in after-ripening. In the latter case there are also found alterations in the acidity and water-holding power of the embryo. Recently, PUGLIESE (55) has investigated the autolysis of oat seeds which had not germinated, and concludes that ungerminated ones may be distinguished from the germinated ones by the presence or absence of amidases or enzymes caring for the end products of the proteolytic digestion. If such enzymatic differences exist between seeds which have been germinated and those which have not, there is quite possibly a very worthy field for investigation in the enzymatic differences between the freshly harvested and the after-ripened seeds, although these differences may not be associated with the problem of dormancy.

It is evident that the conflicts of opinion and the apparent conflicts of fact must be harmonized, if at all, only in the light of careful quantitative determinations of the various factors involved, under standard conditions, and with uniformity of experimental material.

III. Experimental

The work described in this paper has been carried on with seeds of *Avena fatua* received from the Dominion Experimental Farm at Indian Head, Saskatchewan, of the crops of 1910, 1911, and 1912. Parallel tests have been made with seed from Grand Forks, North Dakota, from Brandon, Manitoba, and with seed raised in Chicago

in the summers of 1912 and 1913.[1] Comparisons have been made in many cases with the varieties of *A. sativa* known as Lincoln, Swedish select, Kherson, and White Tartar.

1. GERMINATION TESTS.—Germinative tests made through a considerable period showed for the wild oat seed tested the same progressive increase in percentage of germination which has been noted by other investigators for various grasses. From many tests made those given in table I are typical, A and B representing two varieties.[2]

TABLE I

WILD OATS HARVEST OF 1911

TESTED	SHELL COATS ON		SHELL COATS OFF		IN SOIL	
	A	B	A	B	A	B
December	0.5	4.3	48.0	35	.....	.....
January	.....	.....	70.9	.....	64	.....
March	29.0	.....	90.0	.....	94	.....
April	.....	52.0	.....	84	.....	77
June	60.0	26.0	81.0	86	.....	.....
August	37.0	30.0	93.9	79	96	63

Germination tests were made in Petri dishes on moist absorbent cotton at a temperature of about 20° C. Soil tests were made in the greenhouse, even watering being accomplished by the porous clay cup method (**34**). As pointed out by ZADE, the germination is much better with the shell coats removed. However, there is a rise in germination rate apparent through the after-ripening period by seeds from which they have been removed. The peculiarities of germination are thus not to be attributed to low vitality of the seed used, nor to exclusion effects of the shell coats.

In meeting the problem of after-ripening, it is evident that changes must take place during this period either in the structures inclosing the embryo or in the inclosed members themselves. Tests were undertaken designed to furnish data on this situation. The seed coat of *Avena fatua* is formed by the combined cell layer

[1] For these samples I am indebted to Dr. M. A. BRANNON of Grand Forks, Mr. ANGUS MACKAY of Indian Head, and the Dominion Farm at Brandon.

[2] The term "shell coat" is used in this paper to include the palea and lemma inclosing the seed as opposed to the true seed coat.

remnants of the original ovary wall, the integuments, and the epidermis of the nucellus (**14, 32**). This coat cannot be removed from the endosperm and embryo without injury, and thus attention must be called to the difficulties which are immediately encountered in studying the coat effects of the oat. To know absolutely what the coat effects are is impossible; for to break the coat involves dangers of "wound effects," or of infection; while if the seeds are subjected to fluids or gases, it cannot be said with certainty that any results obtained are due primarily to the external

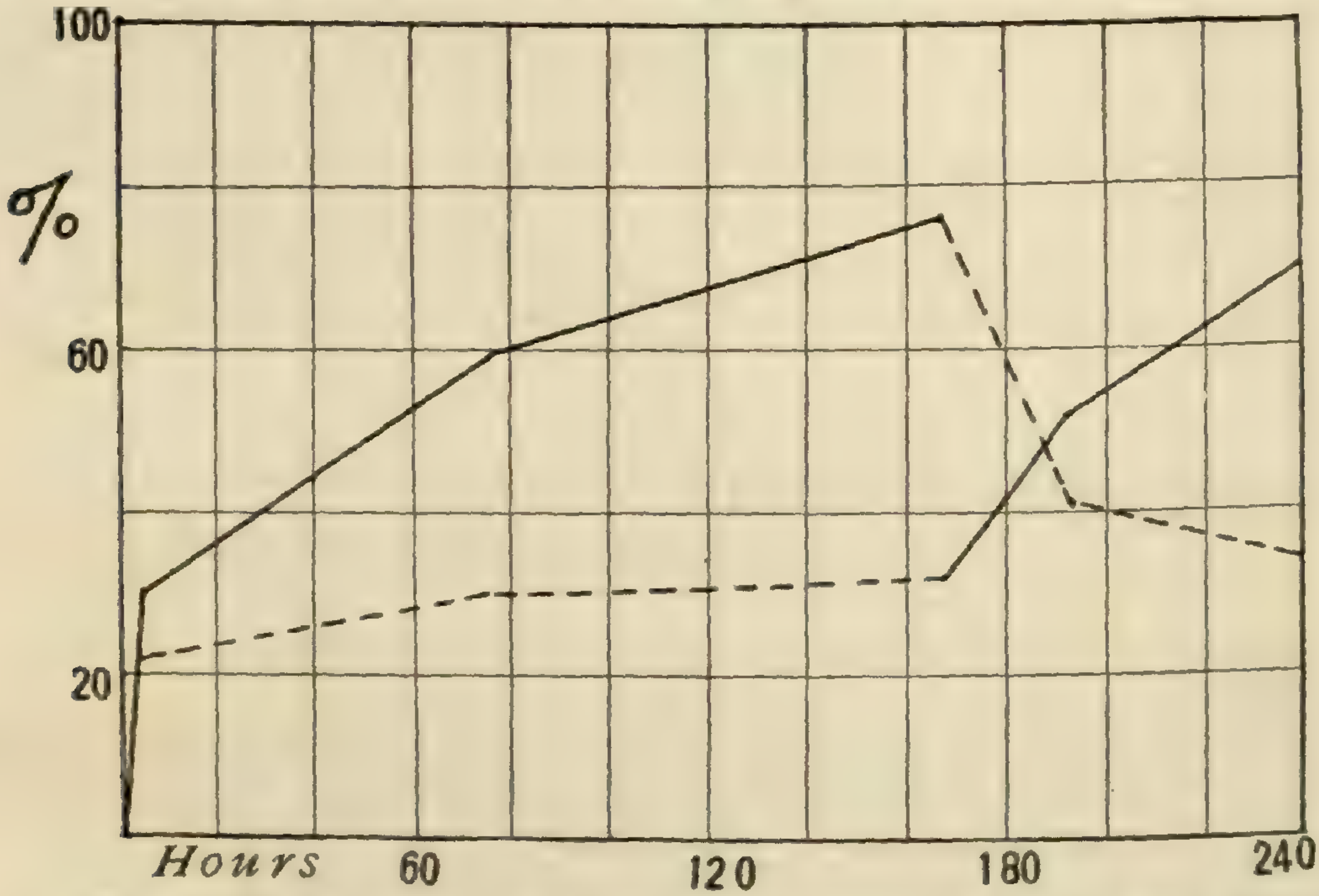

FIG. 1.—Water intake, showing semipermeability of the wild oat; increments in percentage of air dry weight; in water, solid line; in gram-molecular sodium chloride solution, broken line.

agents used, as questions of their actual entry to the embryo arise, and also the question as to what exact physiological function is set in action by such agents, even if they are successful in forcing germination. These difficulties are of course obviated in experimentation with seeds which, like *Xanthium* (**60**), permit of an easy removal of the seed coat. However, in a study of *Avena* it is necessary to gather as much data as possible from a variety of external factors, and, recognizing the uncertainty to which any one line of inquiry alone leads, to judge the situation from the combined results.

2. WATER INTAKE.—BROWN'S work on the semipermeability of *Hordeum* led to like tests of *A. fatua*. Distilled water and gram-molecular solutions of sodium chloride display on test a rather close approximation to perfect semipermeability (fig. 1). Later work by SHULL (**61**) in this laboratory shows that the power of excluding various salts in solution by non-living membranes is a rather common property of seed coats. It was thought possible that there might be some correlation between this behavior of *Avena* and gaseous or water exclusions in unafter-ripened seeds.

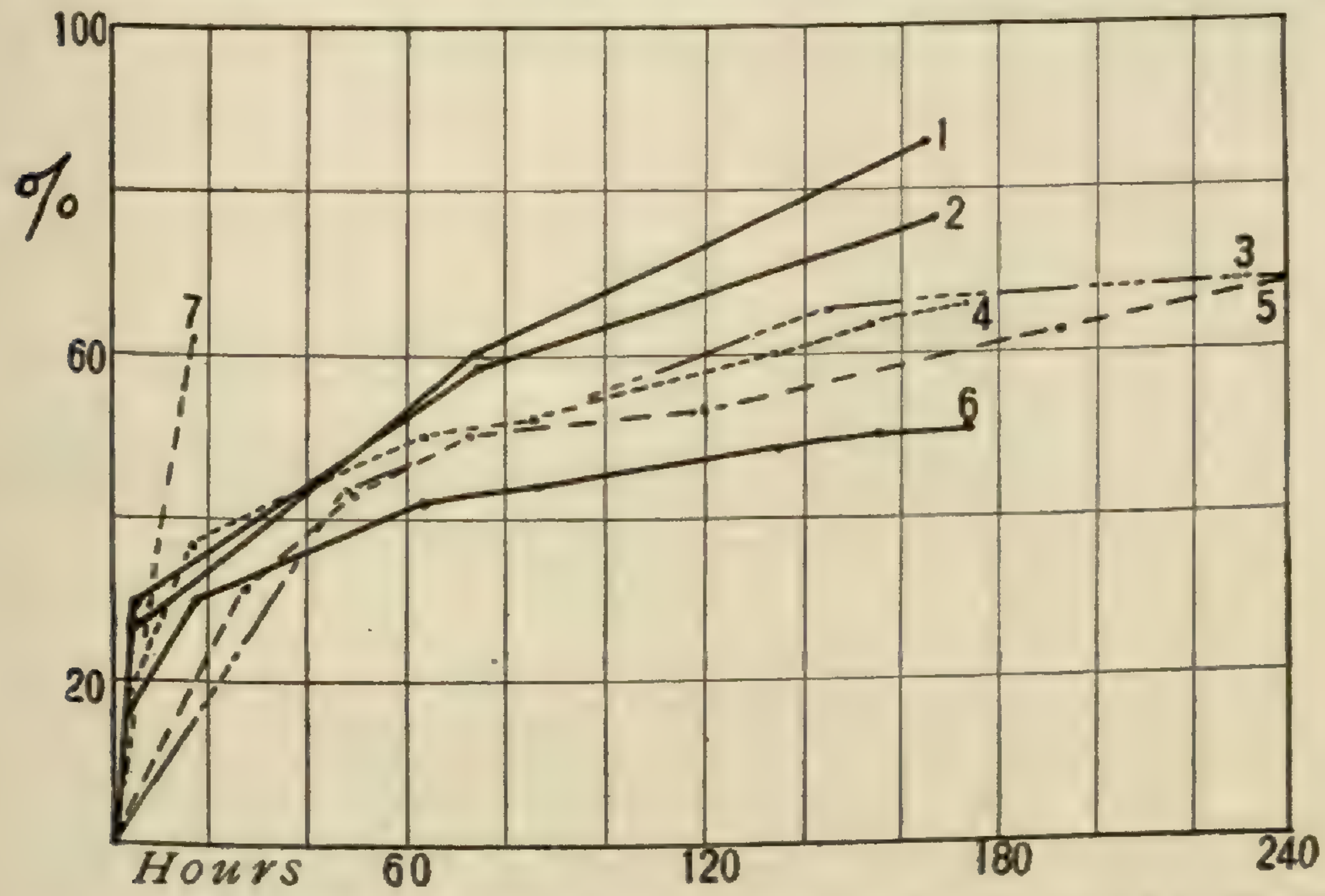

FIG. 2.—Comparative water intake among the Gramineae and in *Xanthium;* increments in percentage of air dry weight; curves: *1*, wild oats 1911; *2*, wild oats 1910; *3*, barley; *4*, *Avena sativa; 5*, wheat; *6*, wild oats 1911; *7*, *Xanthium*.

Tests were thus made of the rate of water intake for seed of different crops and for *A. sativa*, the shell coats being removed in all cases (fig. 2). Comparing the data derived with figures given by SCHRÖDER for *Triticum* and by A. J. BROWN for *Hordeum*, there is seen to be a general similarity in the rate and total water intake for these grasses. Although much slower than the water intake of some other seeds, as for instance *Xanthium* (**17**), there does not seem to be any ground for saying that water exclusion in the case of *A. fatua* can explain its peculiarities of germination in the light of the general behavior of *A. sativa*, *Triticum*, and *Hordeum*.

3. WOUNDING.—Various attempts were then made to see if the slowness of germination soon after harvest could be influenced by other external factors. Tests of light and darkness did not seem to indicate for the wild oat the importance ascribed to these factors by German investigators for other seeds. Using a sterilized needle and pricking the true seed coat of seeds from which the shell coats had been removed tended to raise the germination percentage as pointed out by several other workers. The results given in table II were typical.

TABLE II

No. tested	Month	Per cent germination	
		Pricked	Check
100	December 1911	100	35
100	December 1912	97	48

As regards the relation of seed wounds and water intake, COUPIN (15) has shown that wounds increase the speed of intake, but have no influence on the maximum absorbing power. In order to accomplish the same result of breaking the seed coat without so much danger of infection, the method was adopted of searing the seed near the embryo with a red hot needle. Similar increases in germinative percentage were again noticed. To determine whether the modified behavior of seared seeds could be attributed to "wound effects" of a temporary character, dry seeds were seared, and after a month compared with other seeds previously soaked in water for 24 hours in the ice chest and then seared just before placing in the germinator. No marked difference could be noted in the two lots. Typical results of searing the seeds of *A. fatua* are shown in table III.

TABLE III

Time	No. seeds	Per cent germination	
		Seared	Check
February 1912	100	95	74
March 1912	100	100	90
December 1912	100	99	52
December 1912	100	95	64
January 1913	100	100	67
January 1913	100	98	68

It will be noticed that the difference between the checks and the treated seeds becomes increasingly less conspicuous with the passage of time after harvest, that is, as after-ripening progresses. Tests were made of fresh seed hardly yet "out of the milk," which tend to show that the seed is then neither lacking in vitality nor under the necessity of a long process of drying to secure "necessary protoplasmic alterations." One test (table IV) will show the tendency.

TABLE IV

No. tested	Period	Per cent germination	Then	Additional percentage	Total percentage
50	4 days	28	Seared 3 days.....	70	98
50	4 "	36	In 40 per cent O_2 3 days.....	34	70

In this connection it is interesting to note that ZADE found that seed harvested unripe yielded a higher percentage of germination than seed harvested after ripening. It has been suggested (**49**) that seeds sometimes grow better just before ripening due to the presence of enzymes and protein-splitting products which decrease in amount with ripening as proteins are stored. However, if coat restrictions be concerned in the delay of after-ripening, it is possible that ripening tends to increase the impermeability of this coat. It was also found that improved germination could be secured by complete separation of the embryo from the endosperm. Other workers have successfully grown seeds thus mutilated (**30, 65**). The results secured with *A. fatua* may be illustrated by the instances given in table V.

TABLE V

Time	No. tested	Embryos alone per cent	Check per cent
December 1911.....	100	87	35
December 1912.....	100	87	48

4. RESPIRATORY RATIO.—In considering the effect of breaking the seed coat and its relationship to germination, the question arose as to whether if the seed coat acted as a restriction to oxygen entry, it might not be possible that after-ripening consists in a developing ability of the seed for anaerobic respiration. This was found not

to be the case. The results obtained, however, were interesting in another way. In making these tests, 25 seeds with like control were soaked over night in distilled water in the ice chest, and then placed in inverted test tubes over mercury and kept in the dark at a quite evenly registered temperature of about 21° C. Seeds were allowed to remain about four hours, a period during which the oxygen content of the tube was found not to fall below 16 per cent. At the end of the period the gas was drawn off and analyzed for carbon dioxide and oxygen with a Bonnier and Mangin gas-apparatus in the customary manner. From this data the respiratory ratio $\frac{CO_2}{O_2}$ was computed. Tests were made for the seeds intact, seared, and intact but in 93 per cent oxygen. Anaerobic respiration apparently did not increase in the three months (January to March 1913) during which these tests were being carried on. The point to these results, however, may be noted on taking the average of many determinations made under the three conditions given in table VI.

TABLE VI

	Ratio average
Seeds intact in pure air.....	0.800
Seeds seared.....	0.649
Seeds in 93 per cent oxygen.....	0.557

It is interesting to note the decrease of the ratio as the seeds are seared, and an even greater decrease as they are subjected to increased oxygen concentrations. The results do not seem to be out of harmony with the conception of the seed coat acting as a restriction to oxygen entry.

5. GERMINATIVE TESTS UNDER VARYING CONDITIONS OF OXYGEN.—If the coats should thus tend to exclude oxygen, we might suppose that seared seeds would be able to germinate in an atmosphere of reduced oxygen content as well as intact seeds in air; while intact seeds, it might be supposed, would be benefited in germination percentage if placed in an atmosphere of increased oxygen content. To test out these hypotheses, the seeds were placed on moist absorbent cotton in dishes on tripods, and covered by an

inverted battery jar set in a water seal so as to leave the seeds 2.25 liters of gas, which was introduced by displacement. All was

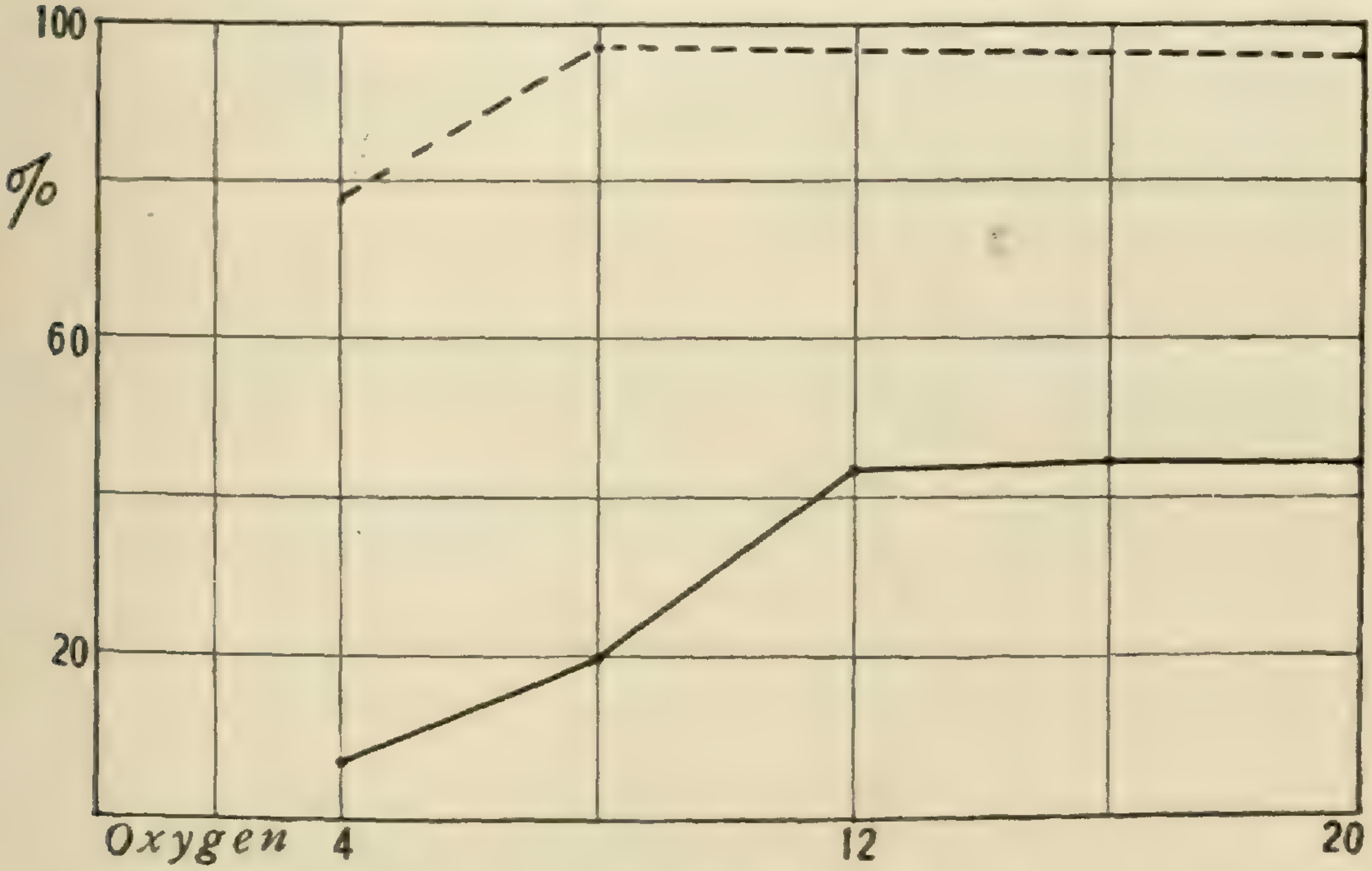

FIG. 3.—Percentage of germination of wild oats in reduced oxygen concentrations; intact seeds, solid line; seared seeds, broken line.

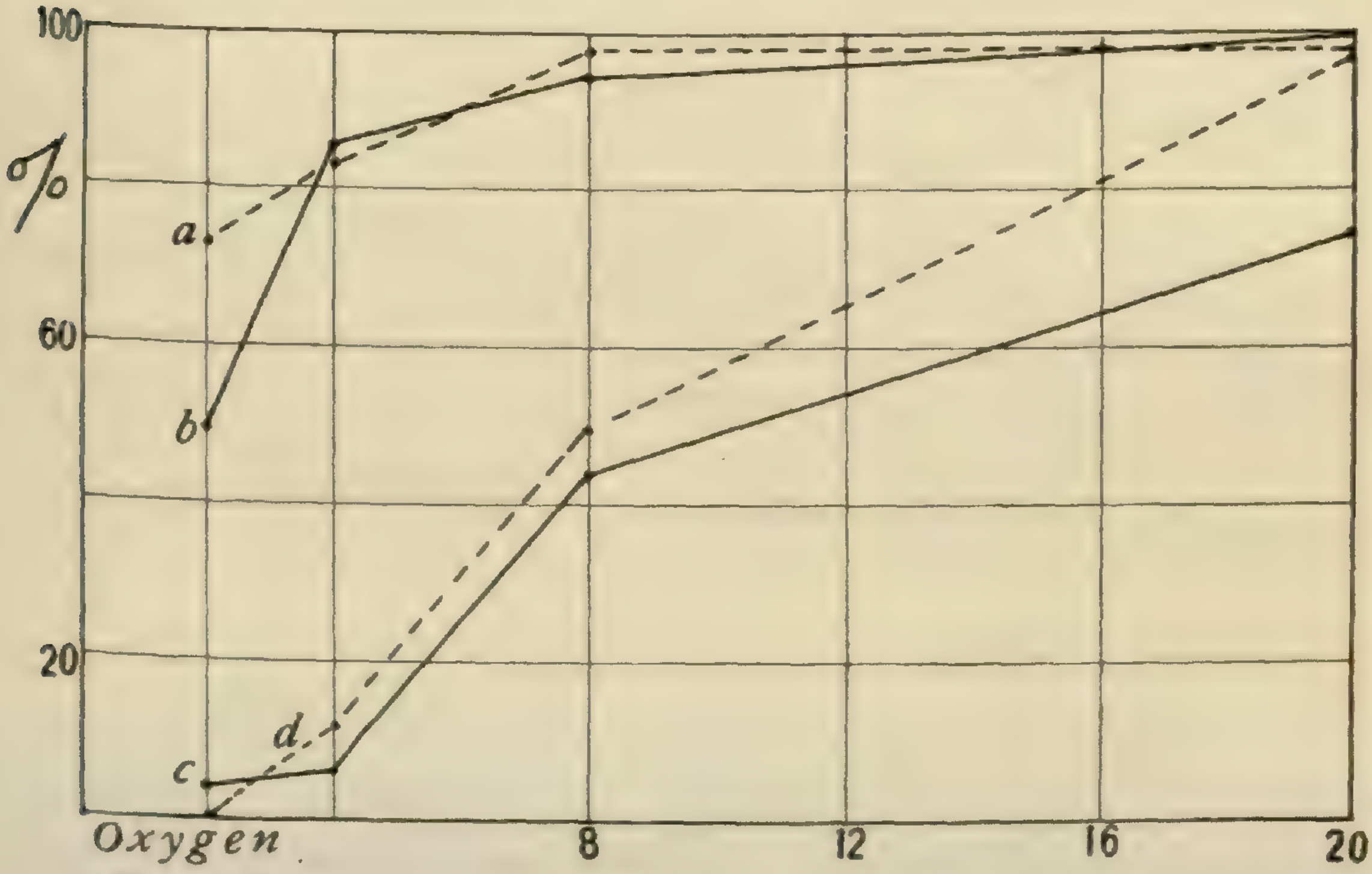

FIG. 4.—Comparison of germination percentages of tame and wild oats in reduced oxygen concentrations; intact seed, solid line; seared seed, broken line; *Avena sativa*, curves *a* and *b*; *Avena fatua*, *c* and *d*.

placed in a dark room at about 20° C. For oxygen content less than the approximate 20 per cent of air, hydrogen was used as dilutant; while for increased oxygen content, the gas was added

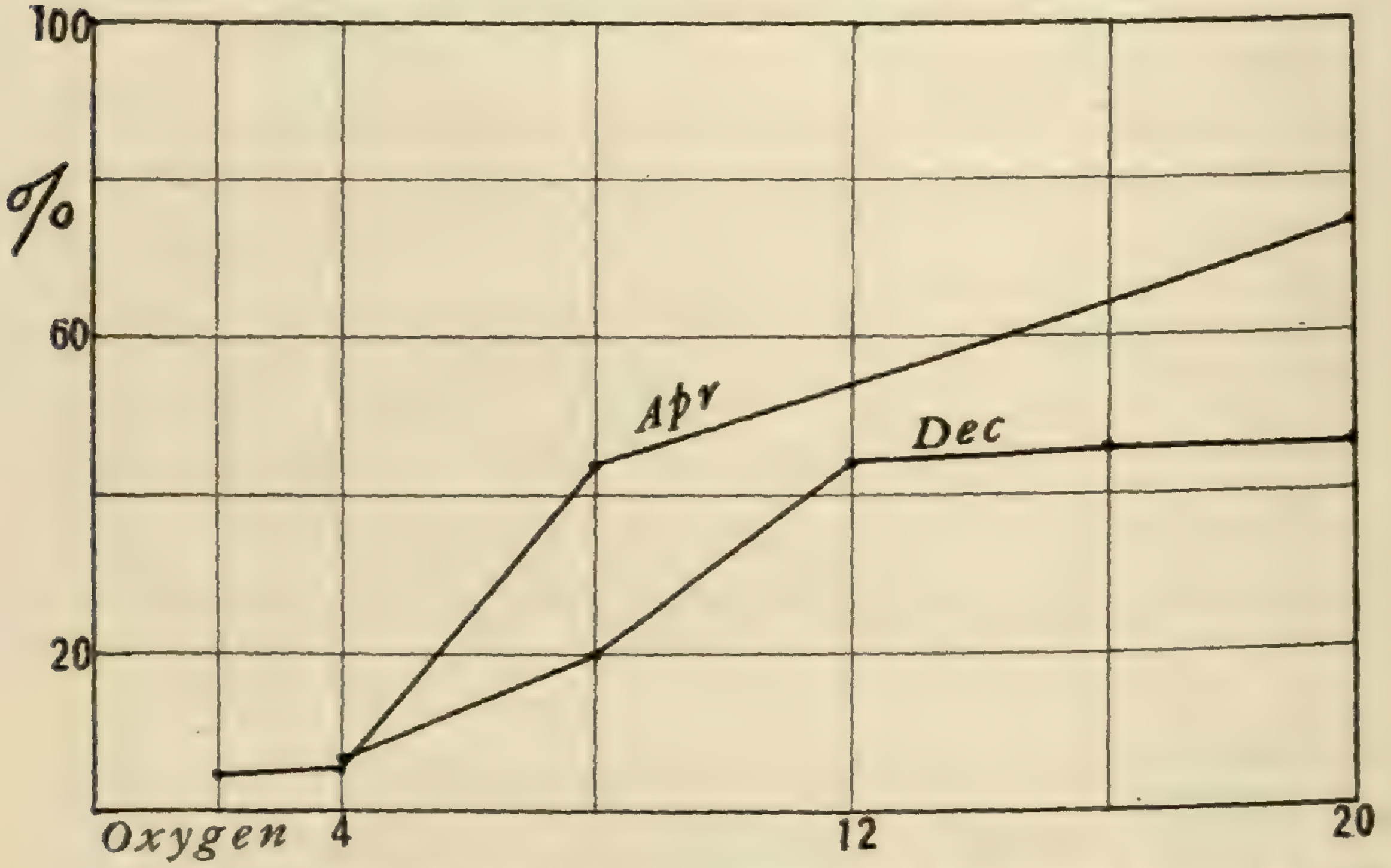

FIG. 5.—Comparison of germination percentages of wild oats in reduced oxygen concentrations in autumn and spring.

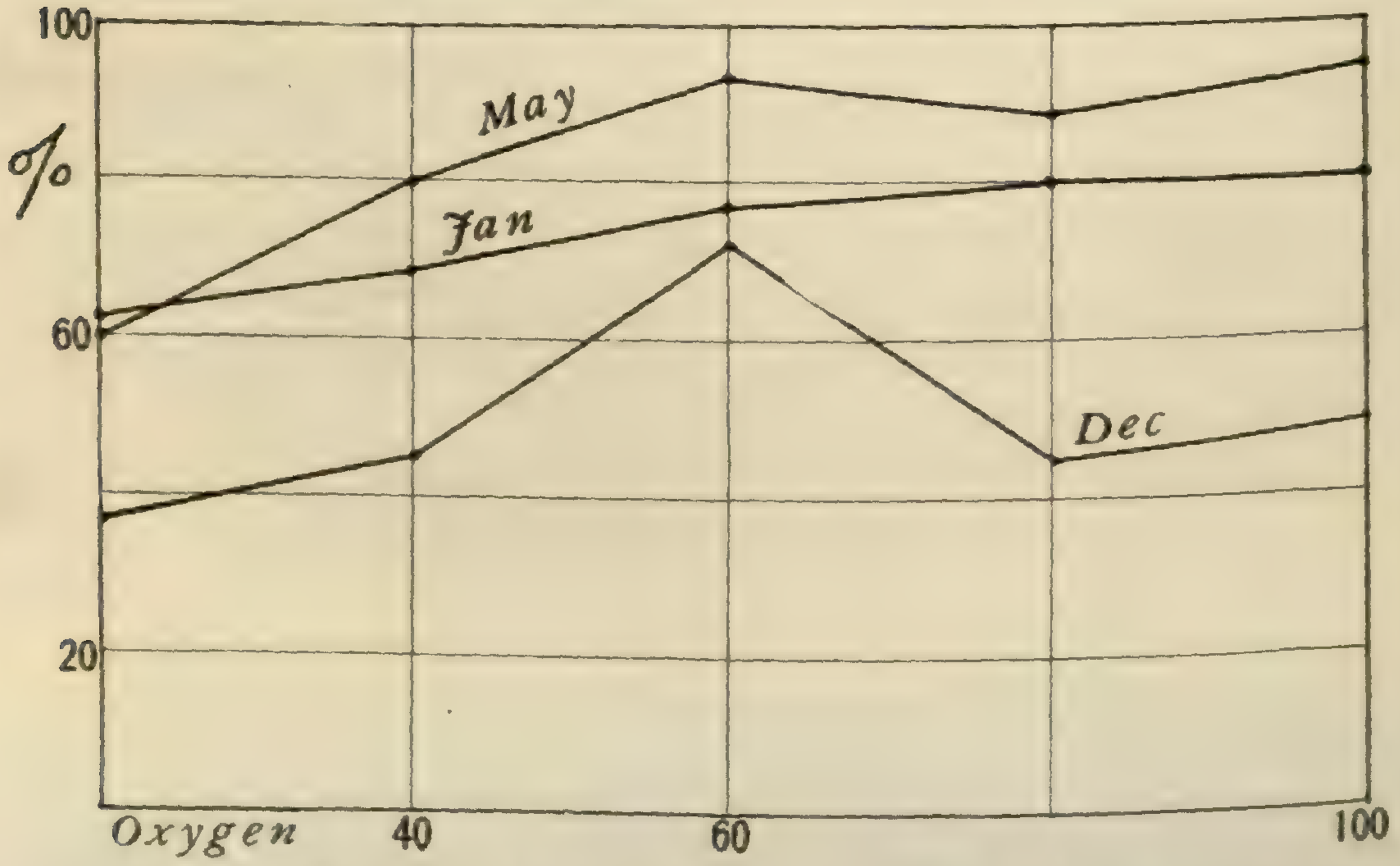

FIG. 6.—Comparison of germination percentages of wild oats through after-ripening period in increased concentrations of oxygen.

directly. The gas used was generated electrolytically by the American Oxhydric Company of Milwaukee, and showed high percentage of purity on analysis. Considering the air check as 20 per cent oxygen, tests were made at the further percentages of 2, 4, 8, 16, 40, 60, 80, and approximately pure oxygen. Comparing the effect of reduced oxygen content on the germination of both intact and seared seeds of *A. fatua*, greater ability is noticed for the seared seeds to grow with good percentages in concentrations of one-fifth the normal oxygen during December (fig. 3).

For April and May, if we compare the sound and seared seeds of *A. fatua* and *A. sativa* as shown in fig. 4, it is evident that searing increases the germination of both at all percentages of oxygen, the difference caused by searing being greater for the wild oat. The tame oat shows a high germination rate even in very low concentrations of oxygen. If we follow the intact wild oat through the winter, there is a noticeable rise in the ability of the seed to grow at lowered oxygen concentrations (fig. 5). If, on the other hand, increased concentrations of oxygen be tried upon *A. fatua* through the after-ripening period, there is noticeable a larger percentage of germination with increased oxygen concentrations up to about 60 per cent, beyond which point the seedlings tend to become stunted. The rise in viability from December to May is also noticeable (fig. 6).

6. MEASUREMENTS OF OXYGEN ABSORPTION.—The above results with variations in the oxygen concentration and in the season of the year may indicate either that as the season progresses there is a decrease in the embryo requirements for oxygen necessary to secure germination, or that there are modifications taking place in the seed by which the entry of oxygen may be more easily accomplished. To determine the nature of the rates of absolute oxygen absorption under these varying conditions, and as compared with *A. sativa*, a modification of the eudiometer, as devised by CROCKER, was employed (fig. 7). Seven seeds were used in each chamber. They were weighed to 0.1 mg., and moisture content determined in other seeds from the same lot, from which data the dry weight of the seeds used was computed. Before setting up the apparatus, the seeds were soaked over night in distilled water in the ice chest.

The seeds tested were suspended from platinum baskets at *A*, being laid on moistened neutral asbestos fiber, the non-organic substance being used to prevent drying out during the test, and at the same time to avoid danger of respiratory changes. Under the baskets were placed small vessels, each containing 1 cc. of 4 per cent potassium hydroxide solution to absorb any carbon dioxide evolved during the period. Test tube *D* was filled with mercury and could be raised and lowered to balance the mercury column in *C* with that in the test tube. Tube *C* was 15 cm. long and had a volume of 2 cc., the graduations for which were made so that readings could be made to 0.01 cc. When the rate of oxygen intake from the air was to be measured, the gas in the apparatus was previously displaced for several minutes with a current of air forced through 40 per cent potassium hydroxide solution to remove any traces of carbon dioxide. Other gases tested were passed through the alkali and apparatus in like manner. During the course of taking the readings, the apparatus was submerged in a Freas constant temperature water bath electrically regulated and accurate to about 0°.01 C. The volume of the chambers was so selected that variations in temperature would introduce an error so small as to be negligible with the water bath used. Thus, for the right chamber a variation of 0°.1 C. would result in an error of 0.0056 cc., and for the left chamber of 0.0062 cc. Corrections for barometric and temperature variations were made; hence any rise of the mercury column as shown by the corrected readings indicated absolute absorption of oxygen by the seeds tested. From the basis of the dry weight of the seeds used the rate of oxygen absorption per gram per hour was com-

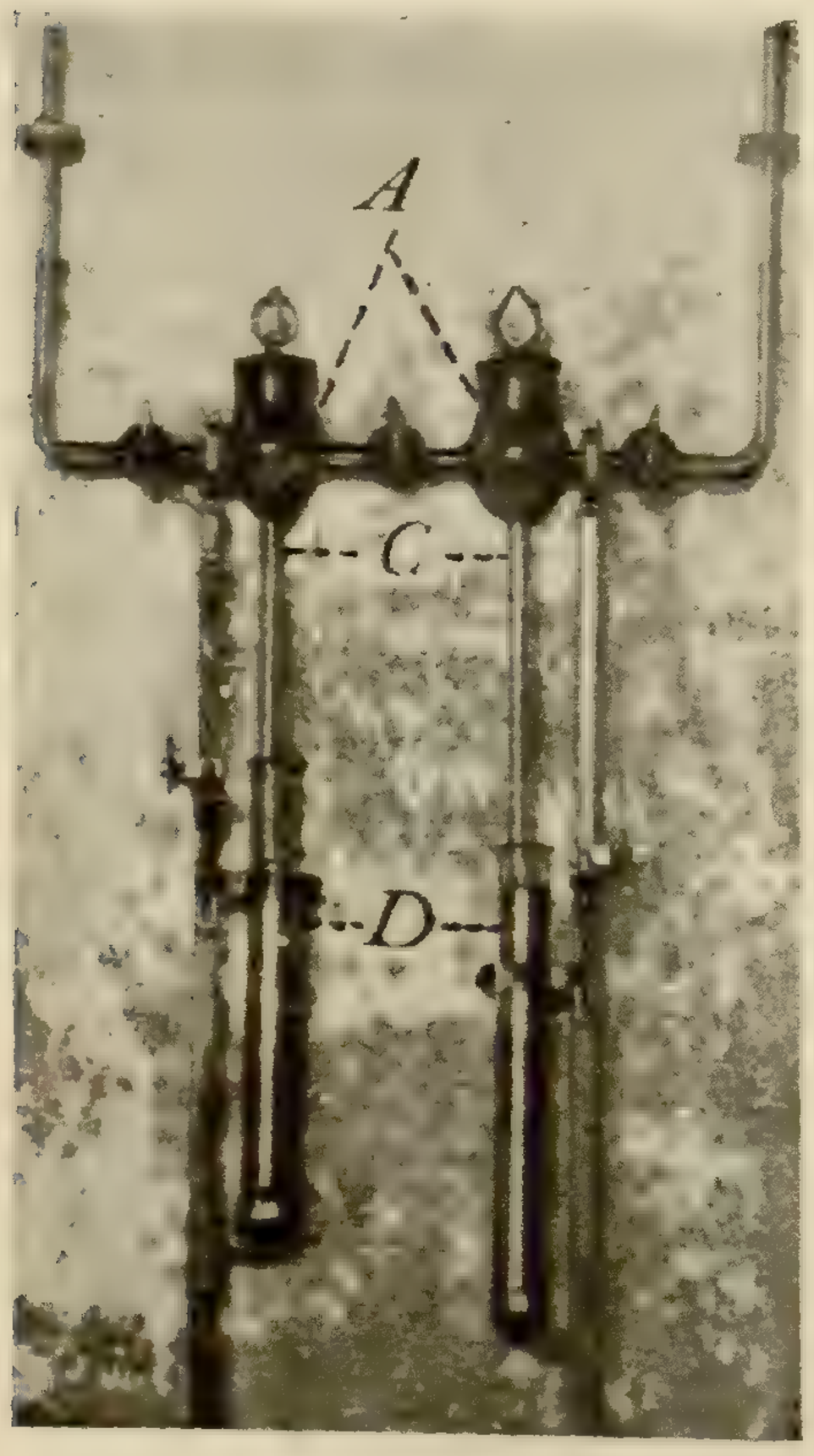

FIG. 7.—Respirometer

puted for the various periods tested. The two chambers served to check all readings. It was found that quite consistent rates were obtained. The rates of oxygen absorption were measured with the bath at the temperature of 21° C.

Before considering the data derived from the respirometer readings, it must be borne in mind that one of the objects sought is to gain a clearer idea of any changes which may occur in the embryo during the after-ripening period. The gas absorption measurements are for the whole seed, of which the embryo forms, by weight, a small part. Hence any variation in the corrected reading for the whole seed might indicate a far greater comparative variation for the embryo. STOWARD (63) has compared the respiration of the separated endosperm and embryo of barley. He finds that per gram of fresh weight, the respiration of the embryo alone is 17 times that of the endosperm, but as the endosperm weighs about 17 times as much as the embryo, these parts probably about halve the respiration of the intact seed. To reason from the respiratory behavior of the separated units to the nature of the respiration of the intact seed seems sufficiently uncertain to justify at least

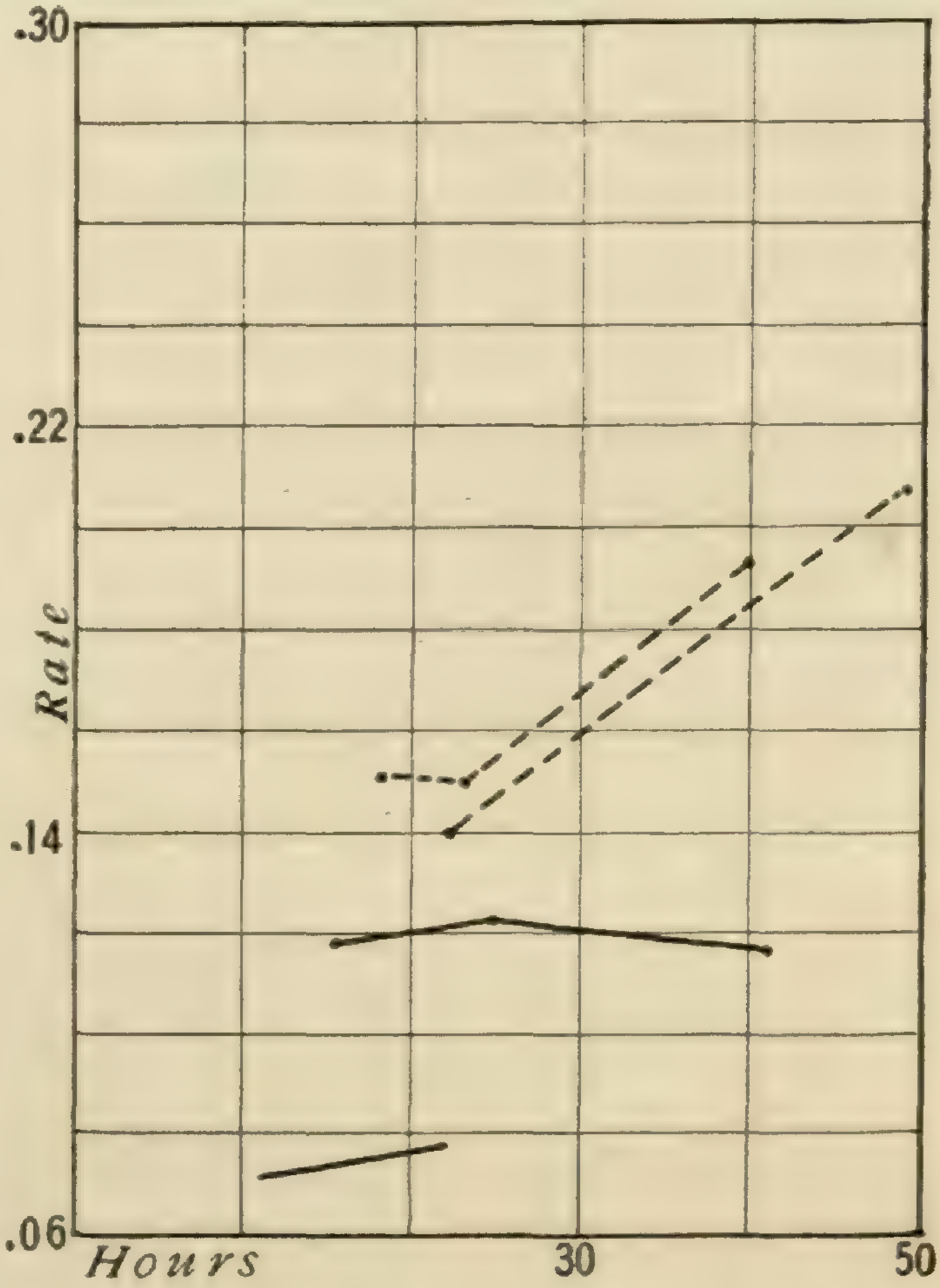

FIG. 8.—Rates of oxygen absorption for wild oats during December and early January in terms of cc. per hour per gm. dry weight; rate for intact seeds, solid line; for seared seeds, broken line.

calling attention to the fact of the combined participation of the embryo and endosperm in the data below.

From a large number of determinations the following will give an idea of the results. Each curve represents the average of the rates derived from both chambers for the periods tested. In fig. 8, curves are shown indicating the absorption rates for intact and seared seeds. These determinations were made during December and early January, when the germination was averaging about 60 per cent for intact seeds. It is evident that the absorption rate is raised noticeably by searing. In most of the curves, a steady tendency is noted for the rate of absorption to rise with the length of the period tested. In many cases the high absorption noted beyond the 25–30 hour periods is associated with the breaking of the seed coats in germination. Earlier increases in rate, however, must not be ascribed to this cause. If now for this same period comparison be made of the absorption rates in the air and in an atmosphere of 93 per cent oxygen, the difference between the two conditions is marked (fig. 9). Further tests in 79 per cent oxygen revealed rates midway between those found for air and for 93 per cent oxygen; while absorption in 7 per cent oxygen

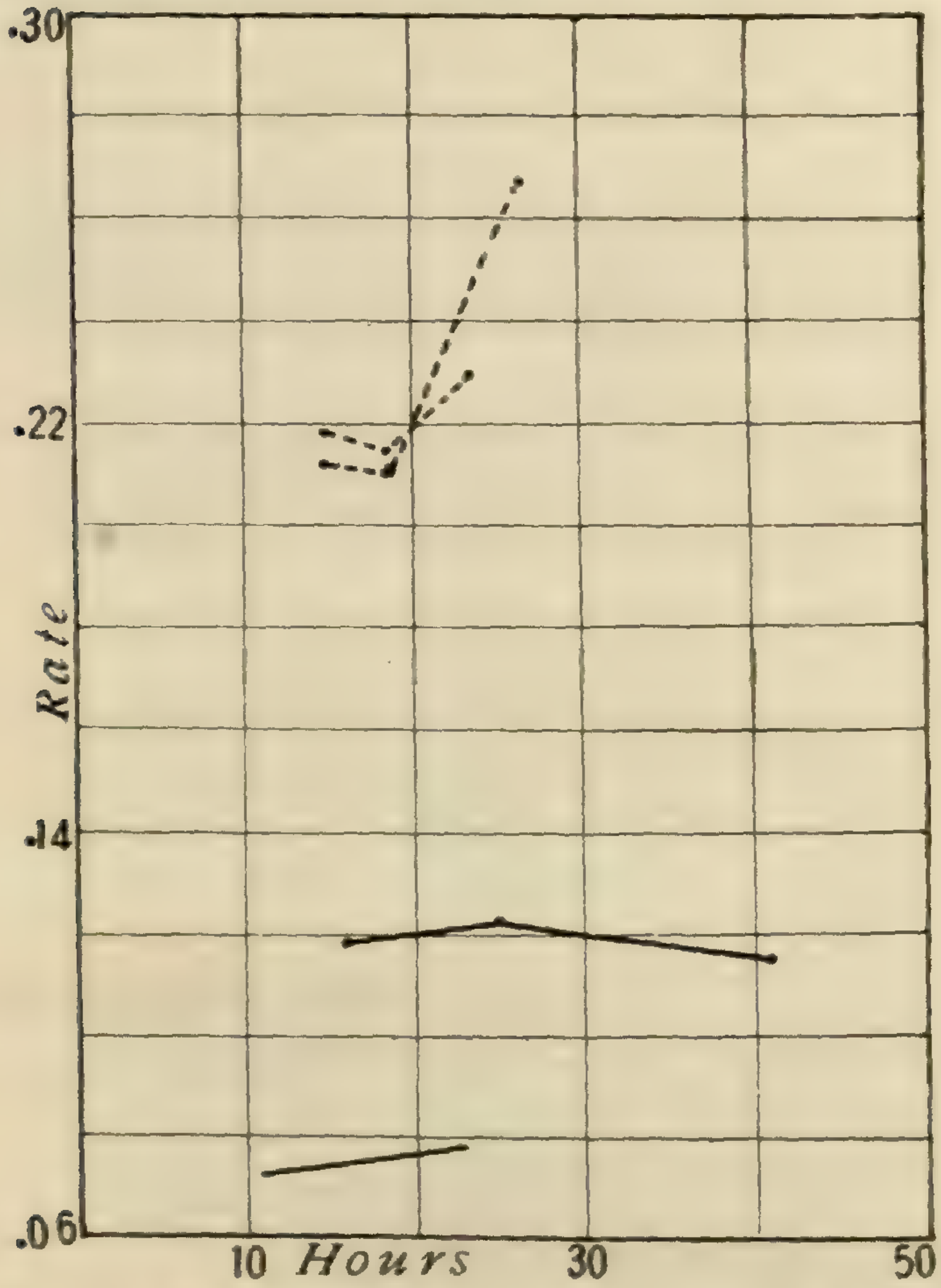

FIG. 9.—Rates of oxygen absorption for wild oats for December and early January in terms of cc. per hour per gm. dry weight; rate for intact seeds, solid line; for seeds tested in 93 per cent oxygen, broken line.

showed much lower rates for the same period than were obtained in air.

Oxygen absorption tests of freshly harvested wild oats were made during July 1913 at temperatures of 16°.2 C. and 26°.2 C. It was thought possible that the failure or success of the VAN'T HOFF temperature law of chemical reaction to hold with the fresh seed might, in connection with the other data, throw some light on the power of the seed coat to exclude oxygen. Summarizing a number of readings, it was found that

$$\frac{\text{the rate at } 26°.2 \text{ C.}}{\text{the rate at } 16°.2 \text{ C.}} = \frac{0.160}{0.067} = 2.38.$$

This is quite what might be expected if the coat offered no restriction to gaseous penetration, and appears at first to conflict with the data derived on this point in other tests. However, we cannot as yet say what effect such a temperature change may exert on the permeability of the coat, which is a non-living structure. It is a well known fact that the solubility of oxygen in water decreases with a rise in temperature. Thus, the absorption at 35° C. is 56.9 per cent of that occurring at 5° C. GASSNER in a recent article (**28**), as reviewed by LEHMANN (**48**), believes that the beneficial results obtained in germination of *Chloris ciliata* through the use of low temperatures may be due to the greater absorption of oxygen at these temperatures. He employed temperatures varying from 5° C. to 34° C. LEPESCHKIN (**50**) and others, however, have pointed out the fact that the permeability of living protoplasm to gases increases with rising temperature. It is thus quite possible that the problem of oxygen absorption by the grasses may be complicated by the opposite influence of high temperature on the solubility of oxygen in the water in the seed, and on the permeability of the seed coat itself. Conclusions on these tests must hence be delayed pending further investigation of the effect of varying temperatures on the permeability of non-living membranes to gases.

As it was noted that in every germination test a large number of seeds laid dormant, yet if forced by searing would promptly germinate, the experiment was tried of testing the oxygen-absorption

rate with seeds selected after lying dormant five days under the usual germinative conditions. The results showed a much lower absorption rate, both in air and in 93 per cent oxygen, than was the case in tests made with unselected seed. Furthermore, the difference between the rates in air and in higher concentrations of oxygen was not so marked. It is realized, of course, that the "selected seed" was not in behavior to be strictly compared with seed treated in the usual manner, as they were subjected to a temperature of 21° C., in the period that germination was attempted before the respirometer test.

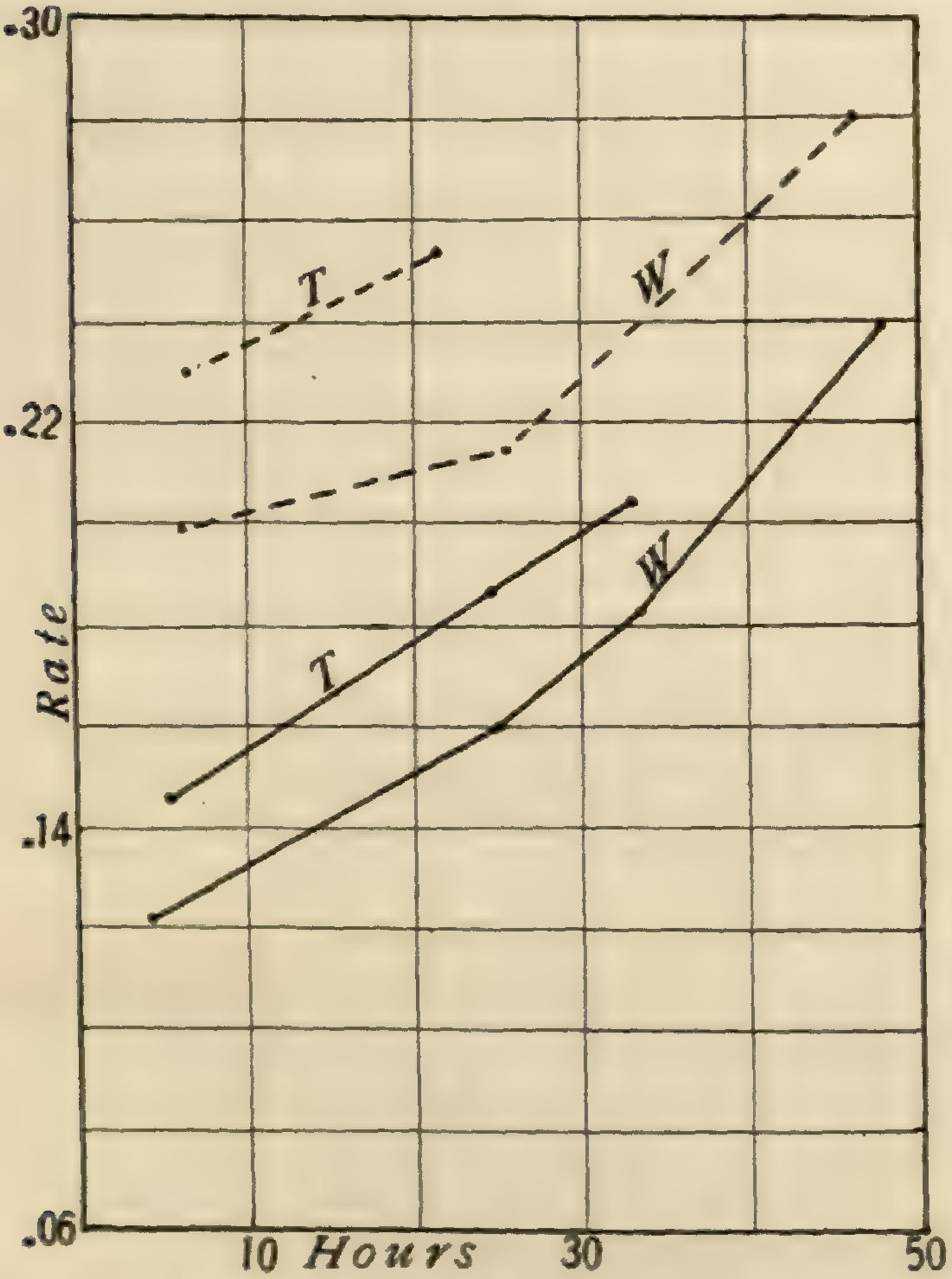

FIG. 10.—Comparative rates of oxygen absorption for tame and wild oats in terms of cc. per hour, per gm. dry weight; *Avena sativa* indicated by *T*, and *Avena fatua* by *W;* rates for intact seeds, solid line; for seared seeds, broken line.

The oxygen absorption seemed to be practically the same for seeds seared dry one month before testing, and those seared after one night of soaking in the ice box, just previous to placing in the respirometer. The similarity in germinative behavior of the seeds treated in these two ways has been noted above. Other workers (**6, 58, 62**) have studied the influence of wounding on respiration as measured by the resultant carbon dioxide releasal. JUNITZKEY (**40**) holds that oxygen absorption and carbon dioxide releasal may be phenomena independent of each other. In any case, we are concerned here only with the direct rate of oxygen absorption by the seed as bearing on the problem of germination. For this

reason it is of interest to note that the temporary "wound effects" noted for other tissues with carbon dioxide as a basis of decision, do not hold for the oxygen intake of *Avena fatua*.

As the early germinative delays of *A. sativa* are so much less pronounced than is the case for *A. fatua*, it is of interest to compare the oxygen-absorption rates for the two (fig. 10). The tame oat shows a higher rate throughout. It was impossible to carry out these tests (summarized in fig. 10) until April, when the comparative difference in germinative delay between the tame and the wild oat is much less than in the preceding autumn. This makes it seem probable that when fresh seed may again be obtainable, the differences in rate found above, though quite marked, may be even more conspicuous.

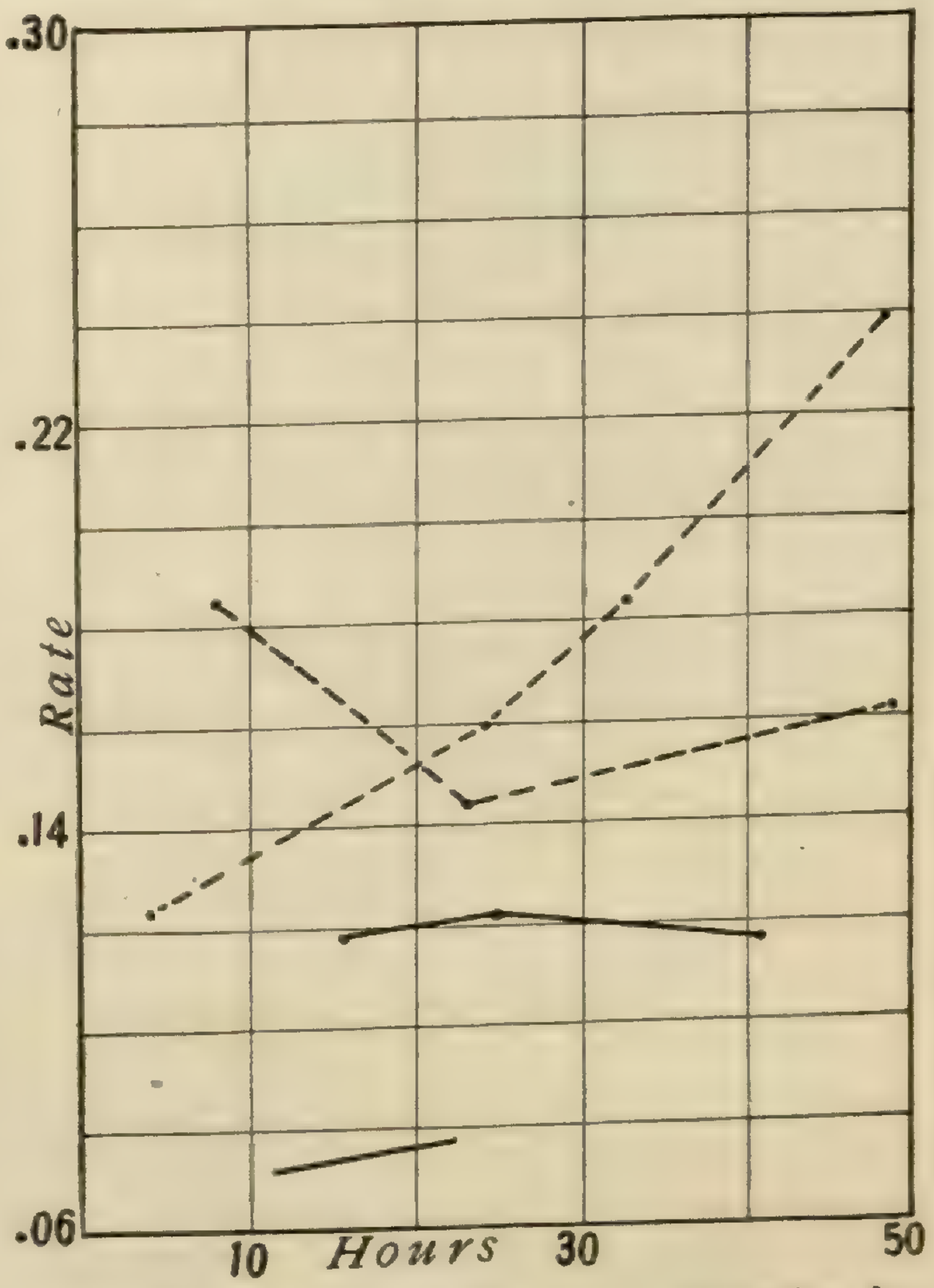

FIG. 11.—Rates of oxygen absorption for intact wild oats before and subsequent to after-ripening in terms of cc. per hour per gm. dry weight; rates in winter, solid line; in spring, broken line.

In figs. 11–13 are shown respectively the effect of after-ripening on the rate of oxygen intake by intact seeds, seared seeds, and seeds run in an atmosphere of over 90 per cent oxygen. The temperatures employed were identical in the 24 tests here summarized. In each case the winter rate is indicated by the unbroken lines, and the spring rate by broken lines. A consistent increase in the rate of oxygen absorption in

higher concentrations is shown. This increase is least noticeable in the case of the high percentage of oxygen tests. It is suggested that if coat restrictions are concerned, and if their effects are overcome by high concentrations of oxygen, the four curves would tend to come together, as is found to be the case.

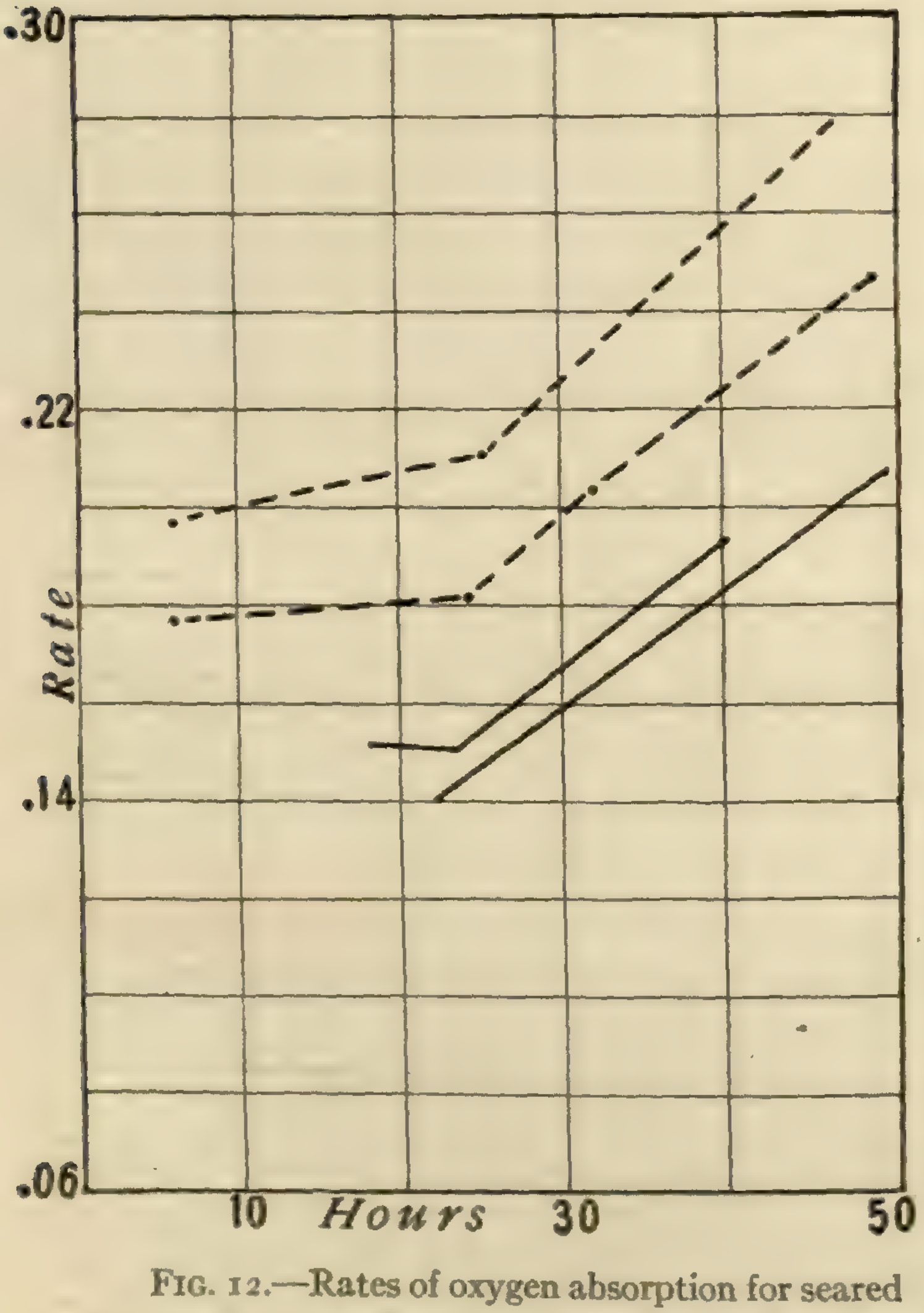

FIG. 12.—Rates of oxygen absorption for seared wild oats before and subsequent to after-ripening in terms of cc. per hour per gm. dry weight; rates in winter, solid line; in spring, broken line.

Summarizing the respiration tests, it would seem that associated with increased germinative rates accompanying after-ripening there is an increased ability of the seed to take up oxygen, providing always that the conditions of germination be the same; and, further, that wounding and subjecting to increased oxygen concentrations actually increases the oxygen intake. Nevertheless, the process of after-ripening may not consist primarily in an increased ability of the embryo to take up oxygen. If the unafter-ripened seeds be freed from limitations to absorption, as was accomplished in the experiments summarized in fig. 12, it is apparent that their oxygen-absorption rate closely approximates that of the seeds which have thoroughly after-ripened. Thus the changes, whether they be seed coat or embryonal, which we ordinarily term after-ripening, and which are exhibited by increased rate of oxygen intake, may be immediately attained by artificially overcoming

the limitations to oxygen entry, if rate of absorption be taken as a basis of judgment.

7. DETERMINATION OF EMBRYO ACIDITY.—Investigation has been begun as to variations in the acidity of the embryo in after-ripening, and comparisons have been made with the embryos of *A. sativa*. To test this condition, the seeds were soaked over night in the ice box, and then subjected to identical germinative conditions for about 18 hours. The embryos were then removed, accurately weighed, carefully ground, and immediately titrated with N/20 alkali in the presence of phenolphthalein. Water content of the embryos was determined on other parallel samples, from which data the dry weight of the titrated embryos could be computed. Figuring then the number of cc. of N/20 alkali necessary to neutralize the acidity of the equivalent of one gram dry weight of the embryos, the results given in table VII were obtained.

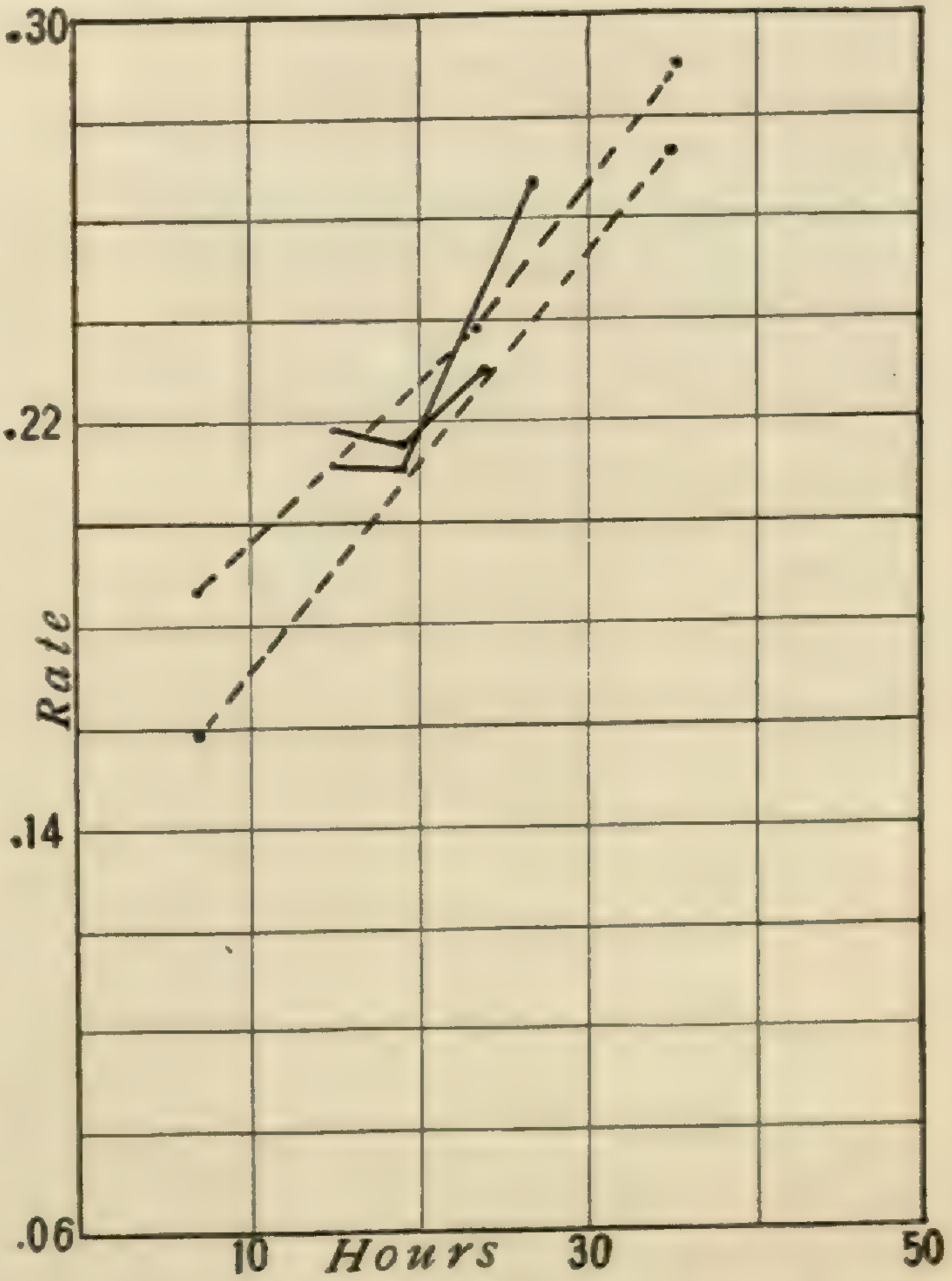

FIG. 13.—Rates of oxygen absorption for wild oats in high concentrations of oxygen before and subsequent to after-ripening in terms of cc. per hour per gm. dry weight; rates in winter, solid line; in spring, broken line.

It will be noticed that the 1912 samples grown at Chicago were tested early and were less acid than corresponding year old samples of both the tame and of the wild. Further, the acidity is less in *A. fatua* seeds one year old than in *A. sativa* just harvested.

Comparing the degree of acidity of the various samples with the moisture contained in the respective embryos, the relations appearing in table VIII are found.

TABLE VII

EMBRYO ACIDITY COMPARISONS OF AVENA FATUA AND A. SATIVA

TESTED AUGUST 1912		
Kind	Season grown	N/20 ALKALI FOR 1 GM. DRY WEIGHT
Tame (Swedish select)	1911	3.70
Tame (Swedish select) tested fresh	1912	2.51
Tame (Kherson)	1911	3.37
Wild (Indian Head)	1911	2.37
Wild (grown Chicago) tested fresh	1912	1.87

It is seen that there is a general tendency for the water-holding power and the acidity to rise contemporaneously. This situation was noted by Miss ECKERSON for *Crataegus*. It is possible that such embryonic changes in *A. fatua* may be causally related to alterations in inclosing structures. Further investigation of the chemistry of the embryo is planned.

TABLE VIII

N/20 alkali to titrate 1 gm. dry weight	Per cent water in embryos
1.87	50.2
2.37	56.0
2.51	54.3
3.37	71.0
3.70	68.3

8. CONCLUSIONS.—The combined results, so far noted, namely, that germination can be increased by various coat-breaking methods; that germination may at all stages be improved by increased oxygen; that when wounded or subjected to increased concentrations of oxygen there is an absolute increase in the rate of oxygen absorption; all seem to point to the conclusion that oxygen supply is for the freshly harvested wild oat the limiting factor to germination, with the probability that coat restrictions to oxygen entry play a rôle. The question still is open as to the nature of the physiological processes for which oxygen is thus essential. GRÜSS (31) believes that in the case of *Phaseolus* the abundant

enzyme content in the cells neighboring a wound occurs as a result of the action of oxygen on the reserve proteins. LEHMANN (49) believes germination stimuli are effective through their influence on protein hydrolysis. The work of Miss ECKERSON and of GREEN suggests the possibility that the development of acidity leads to the liberation of enzymes. What relationship, if any, there may be between acidity, oxygen, enzymes, and germinating power in *A. fatua* is worthy of further investigation.

The character of the changes in the seed of *A. fatua* with after-ripening remains to be discovered.

Oxygen being considered the limiting factor to germination for the freshly harvested seed, it is possible to consider that the embryo in the course of after-ripening either decreases in its demands for oxygen, whereby the seeds become able to grow in gases poor in oxygen; or we may suppose that there is no decrease in oxygen demands, but rather an increased permeability of the coat to oxygen. The fact that after-ripened seeds can grow better than fresh seeds in chambers poor in oxygen, although the former regularly absorb oxygen at a more rapid rate in respiration under normal conditions, together with the results (as shown graphically in fig. 13) that fresh and after-ripened seeds in the presence of high percentages of oxygen absorb at similar rates, seem to favor, but not to prove, the general idea that the coat exclusion to oxygen becomes less complete as the seed after-ripens. What mechanism is released by the greater oxygen supplied either artificially through breaking the coat, or submitting the seed to high percentages of oxygen, or under natural conditions through a slowly developed increase in the coat's permeability to oxygen, is as yet an open question. If further investigation upholds the data given above, showing an increased acidity of the embryo with after-ripening, we must recognize the fact that in *A. fatua* after-ripening involves, in addition to physical changes of the coat, accompanying chemical alterations of the embryo itself.

IV. Summary

1. The germination of *A. fatua* has been found less delayed with the shell coats removed from the seed. However, with the shell coats removed, there exist after harvest germinative delays

which disappear with subsequent weeks. Hence the after-ripening of the seed occurs independent of the shell coats.

2. The after-ripening occurs along with the drying of the seed, but independent of the water content, as air-dried seed soon after harvest yields lower germinative percentages than seeds of similar moisture content the succeeding spring.

3. The germination seems unaffected by light.

4. Exclusion of water by the true seed coat does not seem to explain after-ripening.

5. The delay in germination is occasioned by restriction in the supply of oxygen, which thus acts as a limiting factor to germination. The seed coat is probably an obstruction to oxygen entry. This general situation seems pointed to by the combined results obtained by breaking and searing the seed coat; by removal of the embryo; by germinative percentages obtained in varying concentrations of oxygen, both below and above the normal of the air; by direct measurement of the rate of oxygen intake with intact and seared seeds, and with seeds in varying concentrations of oxygen.

6. The exact nature of the changes in the seed which constitute after-ripening cannot be stated positively. However, the data obtained seem to point to an increased permeability of the seed coat to oxygen, together with a rise in the embryo acid content, which is accompanined by increased water-absorbing power of the embryo.

I wish to acknowledge the cooperation of Dr. WILLIAM CROCKER who suggested the above problem, and has throughout been ready with helpful suggestions. I am also indebted to Miss ECKERSON for bibliographical material, and to my father, Mr. J. R. ATWOOD, for assistance in the tedious labor of preparing the seed for tests.

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UNDESCRIBED PLANTS FROM GUATEMALA AND OTHER CENTRAL AMERICAN REPUBLICS XXXVIII[1]

JOHN DONNELL SMITH

Erysimum Ghiesbreghtii Donn. Sm.—Folia linearia apice calloso acuta deorsum angustata parce calloso-denticulata. Sepala pedicellis bis longiora, lateralia basi saccata. Petala obovata in unguem filoformem attenuata, lamina purpurea. Ovarium acute tetragonum, stylo brevi, stigmate bilobo.

Caules ex eodem rhizomate pluries simplices stricti 4–7 dm. longi rubescentes foliorum costa decurrente striati cum foliis racemo sepalis ovario pube bipartita plus minus strigillosi. Folia inferiora approximata 5–7.5 cm. longa 4–7 mm. lata, superiora sparsa decrescentia, suprema 2 cm. longa 3 mm. lata. Racemi 2.5 dm. longi sulcati, pedicellis 5–8 mm. longis. Sepala lineari-lanceolata 10–12 mm. longa 3 mm. lata costata, lateralia in saccam 1.5 mm. ongam producta. Petála 16 mm. longa, lamina 6 mm. lata saturate purpurea nervosa. Stamina 12 mm. longa. Pistillum petalis aequilonga, stylo 2 mm. longo, stigmate 1.5 mm. lato. Siliqua 3.7 cm. longa, valvis reploque carinatis, seminibus uniseriatis 12–15 immarginatis.

Inter saxa ad declivitates montis, Chiapas, Mexico, Aug. 1864–1870, *August Ghiesbreght*, n. 817.—Inter San Márcos et Ostuncalco, Depart. Quezaltenango, Guatemala, alt. 3000 m., Jun. 1882, *F. C. Lehmann*, n. 1510.

Xylosma chloranthum Donn. Sm.—Spinosum glabrum. Folia lanceolata tenuiter caudato-attenuata basi acuminata crenato-glandulariserrata nitida. Flores feminini umbellato-fasciculati pedunculis medio articulatis paulo longiores. Sepala 4 herbacea pistillo triente breviora. Ovarium tetragynum.

Frutex ad truncum ramosque vetustos spinis digitalibus compositis fuscis uti rami crebre lenticellato-punctatis armatus, ramulorum superiorum virgatorum spinis raris simplicibus tenuibus 6–8 mm. longis. Folia pergamentacea concoloria supra vernicoso-lucida 12–15 cm. longa 3–4 cm. lata ad caudam integra, nervis lateralibus utrinque 6–7, petiolo 5–7 mm. longo canaliculato. Pedunculi indefiniti 2–2.5 mm. longi, bracteis basalibus coacervatis squamiformibus ovatis 0.5–1 mm. longis margine puberulis. Sepala ex cl. repertore viridia ovata 2 mm. longa apice ipso puberula ceterum uti pistillum

[1] Continued from BOT. GAZ. 56:62. 1913.

glabra. Discus leviter crenatus. Ovarium ellipsoideum 2 mm. longum stylo bis longius, placentis 4 biovulatis, stigmatis lobis hippocrepiformibus. Flores masculini et baccae deficientes.

Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m., Apr. 1913, *H. von Tuerckheim*, n. 4111.

Sloanea (§ EUSLOANEA K. Schum.) **Tuerckheimii** Donn. Sm.—Folia ovalia basi acuminata subtus puberula. Racemi singuli simplices, rhachi gracili, pedicellis filiformibus, superioribus 2–3-nis. Stamina sepalis dimidio longiora, antheris quam filamenta bis brevioribus inappendiculatis rima apicali dehiscentibus. Discus intra torum staminalem nullus. Pistillum calyce bis fere longius.

Arbor, ramulis petiolisque teretibus, novellis fulvo-velutinis, denique pubescentibus. Stipulae caducae ignotae. Folia nascentia fulvo-velutina, adulta supra glabra 24–31 cm. longa 13.5–17.5 cm. lata cuspide deltoidea 5–8 mm. longa apiculata in angulum subrectum deorsum desinentia ad apicem versus obscure sinuato-dentata, nervis lateralibus utrinque 11–13 ante marginem anastomosantibus, venis transversis inter se summum 7 mm. distantibus, petiolis 4.5–6 cm. longis apice incrassatis. Racemi ad nodos plerumque defoliatos approximatos siti 8–13 cm. longi pubescentes, pedicellis 1.5–3.5 cm. longis, bracteis bracteolisque lanceolatis 3–5 mm. longis, floribus 1 cm.-diametralibus. Sepala 5–8 sublibera valvata oblongo- vel lanceolato-ovata circiter 4 mm. longa extus pubescentia intus fere glabra, maximo sepius 3-fido. Torus staminalis orbicularis 3 mm.-diametralis foveolatus. Stamina 6 mm. longa, antheris oblongo-ellipticis 2 mm. longis breviter apiculatis, filamentis patenter pubescentibus. Ovarium tetragono-ovoideum 2.5 mm. altum pilosum, stylo ad 1 mm. quadrifido. Capsula juvenilis ellipsoidea 1 cm. longa 0.5 cm. lata spinis 9 mm. longis puberulis setosa, matura ignota.—*S. multiflorae* Karst. valde affinis.

In silvis ad Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m., Mart. et Maj., 1913, *H. von Tuerckheim*, n. 4157.

Ilex (§ EUILEX Loesn.) **costaricensis** Donn. Sm.—Glabra. Folia elliptica breviter acuminata in petiolum angustata et decurrentia coriacea lucida impunctata subtus subtiliter glandulosa integra margine revoluta. Pedunculi solitarii 1–2–3-flori, floribus iso-pentameris. Ovarium ovoideum, stigmatibus sessilibus in hemisphaerium coalitis, loculis uniovulatis.

Ramuli annotini densissime foliati et pedunculi et pedicelli sulcato-angulati. Folia 4.5–6.5 cm. longa 2.5–3.5 cm. lata, nervis lateralibus fortioribus utrinque 7–8, petiolis 1.5–2 cm. longis canaliculatis, stipulis squamae-

formibus deltoideis minutis. Pedunculi axillares vel superiores extra-axillares 8–14 mm. longi, pedicellis singulis vel 2–3-nis 6–8 mm. longis, bracteis stipulae-formibus, floribus tantum femininis notis. Calycis partiti lobi semi-orbiculares 1 mm. longi. Petala subquadrata 3.5 mm. longa atque lata usque ad 1 mm. fere connata. Antherae cassae orbiculares, 1 mm.-diametrales filamento paulo longiores. Ovarium 2 mm. longum stigmatum hemisphaerio bis longius. Drupa deficiens.—*I. mexicanae* (Turcz.) Benth. et Hook. quoad inflorescentiam proxima videtur.

Cuesta dela Cara, haud procul ab El Páramo, Comarca de Puntarenas, Costa Rica, Jan. 1897, *H. Pittier*, n. 10843.

Connarus brachybotryosus Donn. Sm.—Foliola 3 elliptica vel ovato-elliptica cuspidato-acuminata apice ipso obtusa basi rotundata bis longiora quam latiora nitida. Racemi fasciculati petiolo breviores. Sepala petalis paulo breviora staminibus longioribus 3-plo longiora pistillo aequilonga.

Arbor. Folia glaberrima petiolo 6–8 cm. addito 2.5–3 dm. longa, foliolis 1.5–2 dm. longis coriaceis, nervis lateralibus fortioribus utrinque 5 subtus elevatis. Racemi paucifasciculati 4–5 cm. longi cum sepalis ferrugineo-pubescentes, pedicellis 2–3 mm. longis, floribus ex cl. repertore luteo-viridibus. Sepala discreta oblongo-ovata 3 mm. longa. Petala obovato-oblonga 4 mm. longa 1.5 mm. lata glabra. Stamina longiora 1 mm. longa, breviorum antheris subsessilibus. Ovarium lanceolato-ellipsoideum 1.5 mm. longum cum stylo aequilongo appresse pilosum, stigmate 5-capitellato. Capsula ignota.

In silvis ad Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m., Febr. 1913, *H. von Tuerckheim*, n. 4027.

DALBERGIA VARIABILIS Vog., var. **cubilquitzensis** Donn. Sm.—Petiolus communis usque ad 2 dm. longus, foliolis oblongis 8.5 cm. longis 3-plo et ultra longioribus quam latioribus. Cymae fusco-pubescentes 6.5 cm. latae. Stamen vexillare deficiens. Ovarium vacuum.

Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m., Febr. 1913, *H. von Tuerckheim*, n. 4091.

Drepanocarpus (§ RETICULATI Benth.) **costaricensis** Donn. Sm.—Stipulae triangulares spinescentes. Foliola 9–15 oblonga vel elliptica apice late acuta mucronulata basi rotundata supra araneosa subtus fulvo-tomentosa. Flores sessiles, bracteolis calycis duas partes cingentibus. Calycis dentes superiores late rotundati, inferiores anguste triangulares. Vexillum crassum extus sericeum. Stamina iso-diadelpha.

Ramuli juniores cum stipulis petiolis foliolorum pagina inferiore paniculis fulvo-tomentosis, bracteolis calyce vexillo fulvo-sericies. Stipulae 5 mm. longae deciduae. Petiolus communis 10.5–13 cm. longus, foliolis reticulato-nervosis plerumque oblongis 4.5–7.5 cm. longis 1.5–2.5 cm. latis, inferioribus cujusque folii decrescentibus magis ellipticis 3 cm. longis 2 cm. latis, terminali maximo obovato-oblongo. Paniculae in exemplis mancis solum suppetentibus axillares singulae folia superantes, bracteolis subsemiorbicularibus 4 mm. longis persistentibus, floribus 11 mm. longis. Calyx 6 mm. longus, dentibus 2 mm. longis, superioribus 3 mm. latis. Vexillum subcoriaceum suborbiculare 9 mm. longum atque latum alis aequilongum. Stamina carinam cucullatam 7 mm. longam aequantia, vagina jam in alabastro utrinque usque ad basin fissa. Ovarium basi disco cupulari cinctum leviter incurvum villosum uniovulatum, stylo brevissimo. Legumen deficiens.—Ad. *D. salvadorensem* Donn. Sm. floris indole accedens foliis praesertim recedit.

Santiago prope San Ramón, Prov. Alajuela, Costa Rica, alt. 1000–1100 m., Jun. 1901, *Amando Brenes*, n. 14507.—Typus in herbario Musei Nationalis sub numero proprio 861757 servatur.

Lonchocarpus santarosanus Donn. Sm.—Foliola 13–15 lanceolato- vel oblongo-ovata apice tenuiter acuminata basi acuta vel obtusa impunctata. Pedicelli approximati solitarii biflori pedicellis propriis additis florem subaequantes. Vexillum subsemiorbiculare pubescens. Alae et carina rectae exauriculatae. Ovarium biovulatum demum monospermum.

Arbor 6–8-metralis, ramulis novellis petiolulis foliolorum subtus nervis pedicellis calyce ferrugineo-pubescentibus. Petiolus communis 13–18 cm. longus glabrescens. Foliola plerumque 6-juga 4.5–6.5 cm. longa 2–2.5 cm. lata chartacea supra glabra subtus pubescentia, nervis lateralibus utrinque 5–6, petiolulis 4–5 mm. longis. Racemi 6–9 cm. longi supra medium floriferi, pedicellis communibus circiter 4 mm. longis raro 3-floris, pedicellis propriis 2–3 mm. longis infra apicem bracteolatis, bracteis bracteolisque orbicularibus vix 0.5 mm. longis caducis. Calyx late campanulatus 2 mm. longus truncatus. Petala coccinea, vexillo 6 mm. longo latiore quam longiore basi latissime acuto prope unguem transverse bicalloso, alis carinaque late oblongis basi truncatis. Tubus stamineus 4 mm. longus. Ovarium pubescens. Legumen mihi non visum.

Mataquescuintla, Depart. Santa Rosa, Guatemala, alt. 1560 m., Mart. 1894, *Heyde et Lux*, n. 6328 ex Pl. Guat. etc. quas ed. Donn. Sm.

Caesalpinia Bonducella Fleming, var. **urophylla** Donn. Sm.—Petiolus communis 2 dm. longus glaber seta terminatus, foliolis 7-jugis oblongo-ellipticis 6–8.5 cm. longis 3–3.5 cm. latis caudato-

acuminatis seta mucronatis basi rotundatis chartaceis nitidis. Racemus axillaris 22 cm. longus, bracteis patentibus 5 mm. longis, floribus 1.5 cm. longis submasculinis. Calycis tubus obliquus sulcatus 7 mm. longus, segmenta 8–10 mm. longa. Petala nigro-nervosa. Filamenta infra medium villosa.—Exemplum unicum mihi visum quamquam imperfectum varietatem tamen insignem vel forsitan speciem novam constituere videtur.

Ad collem San Isidro prope San Ramón, Prov. Alajuela, Costa Rica, alt. 1300 m., Jul. 1901, *Amando Brenes*, n. 14501.—Typum in herbario Musei Nationalis sub numero proprio 861756 vidi.

Leucaena Shannoni Donn. Sm.—Pinnae 4–5-jugae, foliolis 9–15-jugis oblongis apice rotundato mucronulatis basi rotundatis inaequilateralibus binerviis, superioribus obovato-oblongis basi gibboso-obliquis, petiolo sicut pinnarum rhachis prope apicem glandula oblonga munito. Racemus terminalis elongatus, pedunculis 2–5-nis, floribus glabris.

Petiolus communis 6–8.5 cm. longus teres pubescens. Pinnarum rhachis 6–10 cm. longa lineis duabus pubescentibus a foliolis decurrentibus angulata. Foliola 15–22 mm. longa 5–8 mm. lata pubescentia vel glabrescentia utrinque conspicue nervosa. Racemus 2–4 dm. longus crassus aphyllus glabrescens glanduligerus, pedunculis 12–22 mm. longis pubescentibus apice vel infra apicem bracteis quaternis minutis instructis, capitulis absque staminibus 1 cm.-diametralibus. Calyx turbinatus 2 mm. longus. Petala spatulato-lanceolata 3 mm. longa. Stamina 6 mm. longa, antheris oblongis 1 mm. longis. Stylus 2 cm. longus. Legumen haud satis maturum tantum visum breviter (8 mm.) stipitatum 10 cm. longum 12 mm. latum utrinque acuminatum suturis pubescens, seminibus oblique transversis.

Cojutepeque, Depart. Cuscatlán, Salvador, alt. 900 m., Dec. 1892, *W. C. Shannon*, n. 5032 ex Pl. Guat. etc. quas ed. Donn. Sm.—Eadem planta a *C. B. Doyle* ad Rosario, Chiapas, Mexico, Dec. 1906 lecta, sub numero 87ª distributa, in herbario Musei Nationalis numero proprio 574705 signata exstat.

Pithecolobium (§ SAMANEA Benth.; *Paviflora* Benth.) **adinocephalum** Donn. Sm.—Pinnae 1–2-jugae, foliolis 3–5-jugis lanceolato- vel ovato-oblongis apice ipso obtusis basi acutis centrali-costatis coriaceis supra nitidis subtus puberulis pallidioribus, glandula infra medium petioli oblonga inter foliola parium 1–2 superiorum scutelliformi. Panicula densissime capitulifera, pedunculis radiatim fasciculatis, floribus pedicellatis.

Arbor inermis, coma rotundata, ramulis teretibus glabris. Petiolus communis 2–7 cm. longus cum pinnis 4–7 cm. longis tetragonus glaber, foliolis 2.5–4.5 cm. longis 1–2 cm. latis, nervis lateralibus late patentibus, venis supra conspicuis minute reticulatis. Panicula terminalis 10–14 cm. longa, ramis striatis, infimis 6–9 cm. longis erecto-patentibus, pedunculis 3–6-fasciculatis gracilibus 1.5–2 cm. longis puberulis, capitulis absque staminibus 7 mm.-diametralibus, pedicellis 1 mm. longis puberulis. Calyx campanulatus 1 mm. longus puberulus. Corolla infundibuliformis 3 mm. longa. Stamina in tubum inclusum connata 8 mm. longa. Legumen junius solum visum rectum planum glabrum 1.5 dm. longum 2 cm. latum, seminibus circiter 13.—*Gabiloncillo* incolarum.

Ad fundum *La Verbena* prope Alajuelita, Prov. San José, Costa Rica, alt. 1000 m., *Ad. Tonduz;* Aug. 1894, n. 8932; Dec. 1894, n. 9077.—In silvis collinis, Peninsula Nicoya, Costa Rica, Jan. 1900, *Ad. Tonduz*, n. 13531.

Guamatela Donn. Sm., nov. gen. ROSACEARUM e tribu SPIRAEEIS Focke.—Flores hermaphroditi. Calycis persistentis tubus brevis, segmenta 5 imbricata. Petala 5 ori calycis inserta. Stamina 10 uniseriata segmentis calycinis et petalis opposita, filamentis liberis, antheris cordato-ovatis apiculatis. Discus tubum calycis vestiens concavus margine integro petala et stamina ferens. Carpidia 3 sessilia ope stigmatorum primum connata demum libera, stylis terminalibus, stigmatibus capitellatis, ovulis pluribus suturae ventrali biseriatim affixis ascendentibus. Carpidium seminiferum unicum membranaceum sutura ventrali dehiscens, seminibus pluribus obovoideis exalbuminosis, testa ossea nitida.—Frutex reclinans. Folia opposita simplicia cordato-ovata serrulata palmatinervia. Stipulae liberae setaceae. Racemi terminales, bracteis filiformibus, floribus sanguineis.—Genus foliis oppositis in *Spiraeeis* anormale.—Nomen ope metatheseos syllabarum patriam significat.

Guamatela Tuerckheimii Donn. Sm.—Folia supra glabra viridia bullata subtus niveo-tomentosa 5–7-nervia. Racemi singuli vel bini, pedicellis alternis, inferioribus saepe 3-floris. Calycis segmenta oblongo-ovata acuta. Petala oblongo-elliptica et stamina calyce breviora. Carpidia lanceolato-ovoidea, duobus tertio jam sub anthesi paulo minoribus denique vacuis marcidis. Folliculus subovalis inflatus, stylo incurvo.

Caulis ramosus, ramulis petiolis foliorum utrinque nervis fusco-pubescentibus. Stipulae 2–4 mm. longae. Folia longe tenuiterque acuminata 4.5–9 cm. longa 2.5–5.5 cm. lata, petiolis 0.5–2.5 cm. longis. Racemi

adjecto pedunculo 2.5–4 cm. longo 9–10 cm. longi niveo-tomentosi circiter 10–16-flori, pedicellis 4–5 mm. longis, bracteis binis 6–8 mm. longis. Calycis sanguinolenti tubus 2 mm. longus, segmenta 7 mm. longa striata utrinque incana. Petala 5 mm. longa vix unguiculata nervosa utrinque pubescentia. Filamenta 4 mm. longa e basi superne decrescentia, antheris aegre 1 mm. longis. Carpidia incana sub anthesi 4 mm. longa, stylis rectis 2 mm. longis. Folliculus nondum satis maturus solum suppetens 7 mm. longus 5 mm. latus, seminibus circiter 11–12 imbricatis 1.2 mm. longis atque crassis.

In silvis montanis prope Purulhá, Depart. Baja Verapaz, Guatemala, alt. 1750 m., Oct. 1912, *H. von Tuerckheim*, n. 3903.

Rubus (§ EUBATUS Focke.) **leptosepalus** Donn. Sm.—Foliola quinata orbiculari-ovalia acute caudato-acuminata basi rotundata, ternata ovato- vel obovato-elliptica acuminata basi obtusa vel acuta. Paniculae angustae nutantes laxiflorae superne simpliciter foliatae, bracteis setaceis perlongis. Sepala lineari-lanceolata setaceo-appendiculata petalis suborbicularibus aequilonga.

Rami digitulum crassi subtetragoni sulcati glandulifero-setosi cum ramulis petiolis petiolulis paniculis glandulifero-pilosis recurvo-aculeati rubiginoso-pubescentes. Stipulae petiolares setaceae 10–12 mm. longae. Foliorum inferiorum foliola quinata 10–14 cm. longa 5.5–8.5 cm. lata argute duplo-serrata membranacea supra glabrescentia subtus pubescentia nervo medio aculeata, petiolis 7–8 cm. longis, petiolulo intimo 4.5 cm. longo, extimis 12 mm. longis. Foliorum superiorum foliola ternata 6–10.5 cm. longa 3–5 cm. lata, petiolo 1–2 cm. longo, petiolulis 6–15 mm. longis. Ramuli floriferi erecto-patentes. Paniculae parce minuteque aculeatae rami inferiores breves, superiores subsecundi foliis lanceolatis 4–6 cm. longis interdum suffulti, bracteis 1.5–2.5 cm. longis uti bracteolae 6 mm. longae extus cano-pubescentibus. Sepala appendicula 4–5 mm. longa computata 1.5–1.7 cm. longa utrinque cano-tomentulosa eglandulosa inermia. Petala ex cl. repertore rosea. Receptaculum ovideum 4 mm. longum. Germina numerosa glabra. Fructus desideratur.—*R. adenotricho* Schlecht. habitu affinis sepalis praesertim differt.

Cobán, Depart. Alta Verapaz, Guatemala, alt. 1350 m., Jul. 1912, *H. von Tuerckheim*, n. 2452.

Gilibertia leptopoda Donn. Sm.—Folia integra lanceolata vel lanceolato-elliptica tenuiter acuminata basi acuta. Umbellae terminales racemosae, pedunculis paucis longissimis filiformibus inarticulatis nudis, pedicellis paucis elongatis, floribus 5–6-meris. Calyx turbinatus margine subinteger. Petala triangulari-ovata staminibus paulo longiora.

Arbor. Folia 10.5–13 cm. longa 3.5–4 cm. lata pergamentacea rubro-punctulata, nervis lateralibus fortioribus utrinque 7–8 et venis inconspicuis, petiolis summum 4 cm. longis. Racemi rhachis 2–3 cm. longa, pedunculis 5–7 cm. longis ad apicem rhacheos 4–6-fasciculatis infra apicem 1–4 sparsis sub insertione pedicellorum vix dilatatis, pedicellis 3–8-nis 14–16 mm. longis, bracteis bracteolisque vix ullis. Calyx 2 mm. longus in pedicellum attenuatus. Petala 1.5 mm. longa apiculata striata. Antherae orbiculares 1 mm. longae filamenta aequantes. Discus leviter convexus. Styli in conum brevissimum fere connati. Fructus non suppetit.—Secundum clavem specierum Centrali-Americanarum in BOT. GAZ. **55**:436. 1913 adumbratam haec juxta *G. Rothschuii* Harms locanda discrepat inter alia umbellis paucifloris.

In silvis montanis prope Cobán, Depart. Alta Verapaz, Guatemala, alt. 1550 m., Jan. 1913, *H. von Tuerckheim*, n. 4166.

Faramea (§ TETRAMERIUM DC.) **cobana** Donn. Sm.—Folia lanceolata apice ipso obtusa basi acuminata. Stipulae triangulares aristulatae costa decurrentes. Pedicelli in axibus superioribus fasciculati pedunculo vix ullo insidentes floribus subaequilongi. Calycis eglandulosi limbus denticulatus tubo 3-plo brevior. Corolla triente lobata. Antherae inclusae. Bacca globosa ecostata.

Fruticulus metralis totus glaberrimus, ramulis decursu stipularum bicarinato-angulatis. Folia 5.5–7.5 cm. longa 13–21 mm. lata pergamentacea opaca, costa utrinque prominente, nervis lateralibus patulis utrinsecus 9–11, petiolo 2–3 mm. longo, stipulis e basi crassa 1–2 mm. longa in aristam 3–5 mm. longam sensim productis. Pedunculus 1–2 mm. longus, pedicellis 2–3-nis filiformibus 12 mm. longis. Calyx 2 mm. longus, tubo turbinato, limbo urceolato, denticulis minutis. Corolla (nondum satis aperta solum visa) 12 mm. longa ex schedula coerulea, tubo gradatim dilatato infra medium staminigero, lobis oblongo-ovatis. Antherae subsessiles prope basin affixae 4 mm. longae. Discus limbum calycinum aequans. Stylus 7 mm. longus. Bacca 9 mm.-diametralis saturate atricolor ex cl. repertore, semine sphaerico 6 mm.-diametrali basi profunde excavato, albumine cyaneo.—Juxta *F. pedicellarem* Muell. Arg. locanda.

In silvis montanis oppido *Cobán* vicinis, Depart. Alta Verapaz, Guatemala, alt. 1550 m., Jul. 1912, *H. von Tuerckheim*, n. 2474.

Farameae clavis specierum Centrali-Americanarum.

Sect. I. HYPOCHASMA Muell. Arg.

A. **Folia penninervia.**

1. Calyx denticulatus.....*F. salicifolia* Presl.
2. Calyx inaequaliter lobatus.....*F. eurycarpa* Donn. Sm.

B. Folia trinervia.
1. Folia auriculata.....*F. trinervia* K. Schum. et Donn. Sm.
2. Folia basi acuta.....*F. suerrensis* Donn. Sm.

Sect. II. TETRAMERIUM DC.
A. Inflorescentia corymbosa.....*F. odoratissima* DC.
B. Inflorescentia fascicularis.....*F. cobana* Donn. Sm.

Jacquemontia (§ CYMOSAE Meissn.) **platycephala** Donn. Sm.—Folia cordato-ovata supra glabra subtus minute strigillosa. Pedunculi folia non aut parum superantes. Cyma transversim oblongo-capituliformis compacti-flora imbricato-bracteosa fusco-villosa, bracteis extimis orbicularibus, interioribus oblongo-obovatis. Sepala elliptica acuminata bracteis intimis triente corolla triplo breviora.

Volubilis, caulibus petiolis pedunculis appresse pilosis vel glabrescentibus. Folia 6.5–7.5 cm. longa 4–5 cm. lata mucrone apiculata, nervis lateralibus utrinque 8–9 subtus conspicuis usque ad marginem distinctis, petiolis 2.5–3 cm. longis. Pedunculi 9–13 cm. longi. Cyma (nondum profecto evoluta tantum visa) corollis neglectis 2.5–3 cm. alta 7–9.5 lata, ramis lateralibus brachiatis elongatis, intermedio vix ullo, floribus numerosis subspicatis secundis, bracteis uti sepala extus fusco-villosis intus glabris nigricantibus, extimis duabus 18–20 mm.-diametralibus, interioribus pluribus 17–20 mm. longis 8–10 mm. latis. Sepala cymbiformia 12–13 mm. longa 6–7 mm. lata. Corolla 38 mm. longa ad plicas fusco-villosa ex schedula alba. Stamina ad 10 mm. supra basin corollae inserta 12 mm. longa, antheris 4 mm. longis. Ovarium pilosum, stylo 18 mm. longo, stigmate capitato-bilobo. Capsula ignota.—*Ipomoeae hirtiflorae* Mart. et Gal. et *I. Perryanae* Duchass. et Walp., speciebus ambabus ob inflorescentiam ad *Jacquemontiam* melius ascribendis, nostra valde affinis videtur.

Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m., Apr. 1913, *H. von Tuerckheim*, n. 4133.

Cyphomandra aculeata Donn. Sm.—Omnibus in partibus glabra. Folia solitaria lanceolato-oblonga utrinque acuminata, costa subtus et petiolo aculeatis. Cyma pauciflora. Corolla calyce .3-plo longior rotata. Stamina recta basi connata, filamentis brevibus deorsum dilatatis, connectivo sub antherae apice evanido. Stylus filiformis, stigmate clavato.

Folia in ramulo unico suppetente 8–10.5 cm. longa 2.3–3 cm. lata chartacea, nervis lateralibus utrinsecus 7–8, petiolis 11–14 mm. longis, aculeis minutis recurvis. Cymae unicae vistae pedunculus 4 cm. longus, axes pri-

mariae 7–10 mm. longae, pedicelli 2.5–3 cm. longi, flores 4 circiter 22 mm. longi. Calyx 7 mm. longus, lobis 3 mm. longis semiorbicularibus. Corollae tubus 4 mm. longus, segmenta anguste oblonga 15–16 mm. longa basi 4 mm. lata superne sensim acuteque angustata crassa. Stamina 14 mm. longa in annulo 2 mm. longo connata, filamentis 3 mm. longis triangularibus, antheris anguste oblongis 9 mm. longis, connectivo crasso 2 mm. lato ecalloso. Stylus 6 mm. longus. Bacca ignota.

Prope Cobán, Depart. Alta Verapaz, Guatemala, Apr. 1882, *F. C. Lehmann*, n. 1334.

Brachistus meianthus Donn. Sm.—Omnibus in partibus praeter corollam et stamina glaberrimus. Folia attenuato-acuminata in petiolum oblique attenuata gemina, altero lanceolato-elliptico, altero 2–3-plo minore rhomboideo-ovato. Flores 4–5-meri. Calyx integer. Corollae segmenta tubo intus pubescente bis longiora extus puberula. Antherae cordiformes filamenta pubescentia subaequantes. Bacca coccinea.

Fruticosus, ramulis teretibus. Folia membranacea pellucida minute punctulata integerrima, altero 11–15 cm. longa 3.5–5.3 cm. lata petiole 1.7–2.1 cm. longo, nervis lateralibus utrinque 5–6 sicut venae transversae remotae subtus conspicuis, altero 3.5–6 cm. longo 2–3 cm. lato, petiolo 3–6 mm. longo. Pedunculi pluri-fasciculati nonunquam semel dicotomi, floriferi 12 mm. longi, fructiferi 15 mm. longi. Calyx hemisphaericus 1 mm. altus. Corolla 5–6 mm. longa ex schedula flavicans, tubo ad insertionen, staminum pubescente, segmentis oblongo-ovatis. Stamina 4 mm. longa, antheris acutis. Stylus corollam paulo superans. Bacca calyci immutato insidens globosa 6–7 mm.-diametralis.—*B. hebephyllo* Miers. proximus.

Pansamalá, Depart. Alta Verapaz, Guatemala, alt. 1250 m., Febr. 1887, *H. von Tuerckheim*, n. 1134 ex Pl. Guat. etc. quas ed Donn. Sm.—In silvis ad Panzal, Depart. Baja Verapaz, Guatemala, alt. 1350 m., Oct. 1912, *H. von Tuerckheim*, n. 3936.

Columnea (§ EUCOLUMNEA Hanst.) **cobana** Donn. Sm.—Valde anisophylla. Folia subsessilia lanceolato-oblonga vel -elliptica attenuato-acuminata basi inaequali acuta supra glabra subtus nervis marginibusque pilosa ceterum sparsim strigillosa. Pedunculi folio majore paulo breviores. Calycis segmenta attenuato-ovata dentata. Corolla calycem 4-plo superans, tubo limbum aequante, galea truncata integra.

Fruticulus epiphytalis. Caules subtetragoni radicantes, novelli sicut petioli et pedunculi pilis patentibus articulatis rubro-tinctis hirsuti. Folium in pare majus 6–7.5 cm. longum 1.5–2 cm. latum, alterum 18–30 mm. longum

8–13 mm. latum, nervis lateralibus utrinque 4–5, venis obsoletis, petiolis 1–2 mm. longis. Pedunculi singuli 4–6 cm. longi fuscescentes. Calycis obliqui viridis segmenta 1.5 cm. longa 7 mm. lata pilosa, dentibus utrinque 4 obtusis glandula apiculatis. Corolla coccinea extus pilosa intus rubro-granulifera 6–6.5 cm. longa basi gibbosa, lobo antico triangulari-oblongo obtuso 16 mm. longo 7 mm. lato, lateralibus obtuse deltoideis cum galea ad 13 mm. connatis margine superiore 8 mm. longis, galea propria 18 mm. longa 15 mm. lata. Stamina galeam usque ad 7 mm. superantia, antheris subquadratis 3 mm. longis. Disci glandula unica quadrata 1.5 mm. longa bifida. Ovarium ovoideum 4 mm. altum pilosum, stylo rubro cum staminibus supra medium puberulo, stigmate integro.—Ad *C. magnificam* Klotzsch et Hanst. floribus accedens.

In silvis montanis ad Cobán, Depart. Alta Verapaz, Guatemala, alt. 1550 m., Jul. 1912, *H. von Tuerckheim*, n. 2475.

Columnea (§ EUCOLUMNEA Hanst.) **lutea** Donn. Sm.—Folia leviter disparia subsessilia ovato- vel subrhomboideo-oblonga inaequilatera apice acuta basi obtusa vel retusa integra margine revoluta supra cano-strigillosa subtus nervis pilosa. Calycis segmenta obovato-elliptica nervosa utrinque viridia et cano-villosa. Corolla lutea. Disci glandula minima bidentata.

Fruticosa, caulibus ascendentibus approximatis pluribus teretibus, novellis et pedunculis patenter fusco-pilosis. Folia 2–3 cm. longa 10–12 mm. lata, nervis lateralibus utrinque 4–5, venis obsoletis, petiolis 1mm. longis. Pedunculi singuli 11 mm. longi. Calycis segmenta 1 cm. longa 4 mm. lata. Corolla ex cl. repertore lutea extus arachnoidea intus glabra circiter 6 cm. longa incurva basi leviter gibbosa, lobo antico lanceolato-lineari 24 mm. longo 4 mm. lato, lateralibus cum galea usque ad ejus duas partes connatis margine superiore 6 mm. longis, galea propria 12 mm. longa explanata 15 mm. lata fornicata emarginata genitalia superante. Stamina glabra, antheris 2 mm. longis 1.5 mm. latis. Disci glandula vix 0.5 mm. longa. Ovarium cano-pilosum, stylo complanato bifariam puberulo, stigmatis lamellis ovatis 2 mm. longis. Bacca globosa 7 mm.-diametralis calyce accrescente bis superata, glandula 1.5 mm. longa.—Ad *C. microcalycem* Hanst. floris structura accedens colore abhorret.

Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m., Febr. 1901, *H. von Tuerckheim*, n. 7930 ex Pl. Guat. etc. quas ed. Donn. Sm.

Aegiphila (§ CYMOSAE Schau.; *Subtruncatae* Briq.) **fasciculata** Donn. Sm.—Folia longiuscule petiolata opposita lanceolato-oblonga utrinque acuminata supra bulboso-pilosa subtus ochraceo-tomentosa. Cymae femininae sessiles, axibus obsoletis, floribus fasciculatis. Calyx truncatus 4-mucronulatus. Stamina ananthera.

Arbor, ramulis subtetragonis sicut petioli foliorum subtus nervi bracteae pedicelli calyces ochraceo-velutinis, internodiis superioribus 2–3 cm. longis. Folia 15–22 cm. longa 5.5–7.5 cm. lata tenuiter acuminata medio latissima, nervis subtus elevatis conspicuis, lateralibus utrinsecus 6–7, petiolis 2–3.5 cm. longis. Flores solum femini suppetentes in axillis subglomerati subsessiles 4-meri, bracteis linearibus 6–12 mm. longis convolutis deciduis, pedicellis 1–2 mm. longis. Calyx turbinato-campanulatus 6–7 mm. longus minute mucronulatus. Corolla infundibuliformis 9–10 mm. longa alba cum genitalibus glabra, lobis tubum intus furfuraceum aequantibus ovatis acutis. Stamina ad 2 mm. supra basin tubi inserta subulata 1 mm. longa. Stylus 12 mm. longus triente bifidus, ramis papillosis. Drupa ignota.—*A. tomentosae* Cham. quoad inflorescentiam proxima.

In silvis prope Cobán, Depart. Alta Verapaz, Guatemala, alt. 1350 m., Dec. 1912, *H. von Tuerckheim*, n. 4013.

Aegiphilae clavis specierum Centrali-Americanarum.

I. Cymae axillares.
  A. Cymae sessiles.....*A. fasciculata* Donn. Sm.
  B. Cymae pedunculatae.
    1. Cymae densiflorae.....*A. arborescens* Vahl.
    2. Cymae laxiflorae.
      a) Folia opposita.....*A. odontophylla* Donn. Sm.
      b) Folia verticillata.....*A. anomala* Pittier.

II. Thyrsi axillares et terminales.
  A. Calyx truncatus.
    1. Rami subteretes.....*A. martinicensis* L.
    2. Rami acute tetragoni.....*A. falcata* Donn. Sm.
  B. Calyx lobatus.
    1. Corolla infundibuliformis.....*A. brachiata* Schlecht.
    2. Corolla hypocrateriformis.....*A. elata* Sw.

Scutellaria (§ VULGARES Benth.; *Coccineae* Briq.) **isocheila** Donn. Sm.—Folia lanceolato-oblonga utrinque acuminata vel acuta sinuato-dentata discoloria praeter nervos subtus fusco-puberulos glabra. Racemi foliis breviores densiflori, bracteis lanceolatis, inferioribus pedicello 2–3-plo longioribus, superioribus eo brevioribus. Corolla usque ad fauces graciliter tubulosa aequaliter bilabiata.

Fruticulus erectus semimetralis parce ramosus, ramis subtetragonis cum petiolis racemis calyce fusco-puberulis. Folia 6–8 cm. longa 2–2.5 cm. lata subtus pallida, nervis lateralibus utrinque 4–5, petiolis 8–11 mm. longis. Racemi terminales floribus ademptis 2–3 cm. longi 12–16-flori, bracteis

foliaceis, infimis 8 mm. longis, superioribus minutis, pedicellis 2–3 mm. longis, floribus oppositis secundis. Calyx 4 mm. longus, scutello 2 mm. longo. Corollae roseae pubescentis tubus 2.5 cm. longus, labia 3 mm. longa, lobi erecti integri, laterales cum postico breviter coaliti eum aequantes. Stamina majora 12 mm. longa alteris bis longiora, antheris canescentibus eciliatis, loculis omnibus polliniferis. Nuculae deficientes.—*S. Lindenianae* Benth. ex char. proxima.

In silvis humidis ad Cerro de Carizia, Prov. Heredia, Costa Rica, alt. 1800 m., Sept. 1900, *H. Pittier*, n. 16128.

BALTIMORE, MD.

THE OVARY AND EMBRYO OF CYRTANTHUS SANGUINEUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 186

MARGARET ELIZABETH FARRELL

(WITH PLATE XXIV AND THREE TEXT FIGURES)

Cyrtanthus sanguineus Hook. (Amaryllidaceaė) is one of the 37 species credited to the genus. It was first figured in the *Botanical Magazine* (1), and was copied by PAX (2) in his monograph of the family. The genus is restricted to South Africa. *C. sanguineus* and two other species were procured by Dr. C. J. CHAMBERLAIN from the Botanical Garden at Durban, a garden which, like most of those of the colonies, is supported by the nursery trade. The plants are not raised from seed in the garden, but are brought there after being dug up from places in which they grow naturally. The plants secured by Dr. CHAMBERLAIN have been growing in the greenhouses of the University of Chicago. The first blossoms appeared in the winter of 1912–1913. They were hand-pollinated, and from the ovaries and embryos thus derived the present study was made.

Cyrtanthus sanguineus has a tunicated bulb about two inches in diameter. The leaves are thick and leathery, of a bright green color, and about a foot long. The epigynous flower is 3–4 inches in length, and of a rich coral color, almost crimson. The perianth tube is either suberect or decidedly curved, and the upper half of its throat is about one inch in diameter. The stamens are uniseriate and slightly exserted; the filaments are incurved and the anthers are oblong. The ovary is 18 cm. long and 6 cm. in diameter. The ovules are campylotropous. In an ovary of about 12 ovules only 6 or 7 develop to maturity.

Ovary

The material was taken at different ages, killed in chromacetic fluid, and the sections stained in safranin and gentian. All the drawings were made with the help of an Abbé camera lucida.

The ovary is composed of three carpels. I found two different arrangements of their vascular bundles according to location. In the region of the lateral fusion of the carpels, there are three bundles grouped as one. All are collateral; the outer one is ectophloic, and the other two are so arranged that their xylem masses face each other (text fig. 1). The strands at the midrib of each carpel appear in three distinct groups; the outermost one consisting of a single collateral ectophloic bundle; the innermost one of two bundles with their xylem masses facing; and the intervening group composed of two double bundles, the degree of approximation of which differs at different levels in the carpel. One of the levels is shown in text fig. 2. This peculiarity of arrangement is probably due to the curving of the edge of the carpels to form the closed ovary. A closer study led to an explanation of the different groups of bundles, and showed that each organ of the flower is supplied from these vascular strands. Text fig. 3 is a diagram of the flower, the dotted line representing the outline of the ovary. It can be seen that the abortion of the stigmas occurred opposite the region of the fusion of carpels. On tracing their bundles downward into the ovary, they are found to be derived from the primary groups in the carpel. Lower still, these bundles fuse with those of the flower stem.

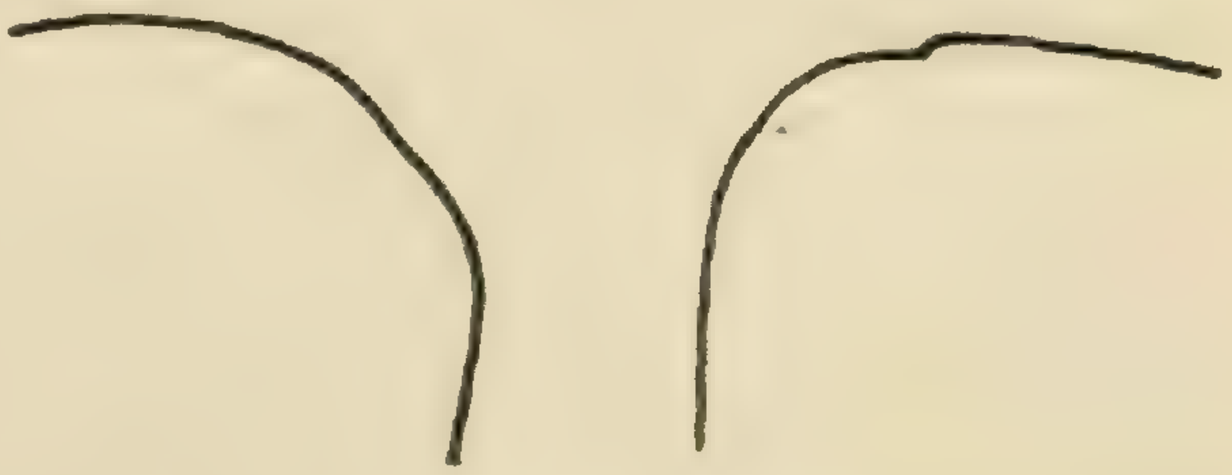

FIG. 1.—Showing the vascular arrangement at the fusion of the carpels.

Noticing the frequent occurrence of stomata on the inner surface of the carpels, I was interested to know the relative number per unit of area in comparison with the outer surface. I found the relation to be as 8:5 in favor of the inner side. This is contrary to what we should expect, for in the foliage leaves of *Cyrtanthus* the stomata are more numerous on the abaxial surface.

Some of the ovules which were killed in the early stages were examined and found to contain an embryo sac of the lily type. The

FIG. 2.—The vascular arrangement at the midrib of the carpel

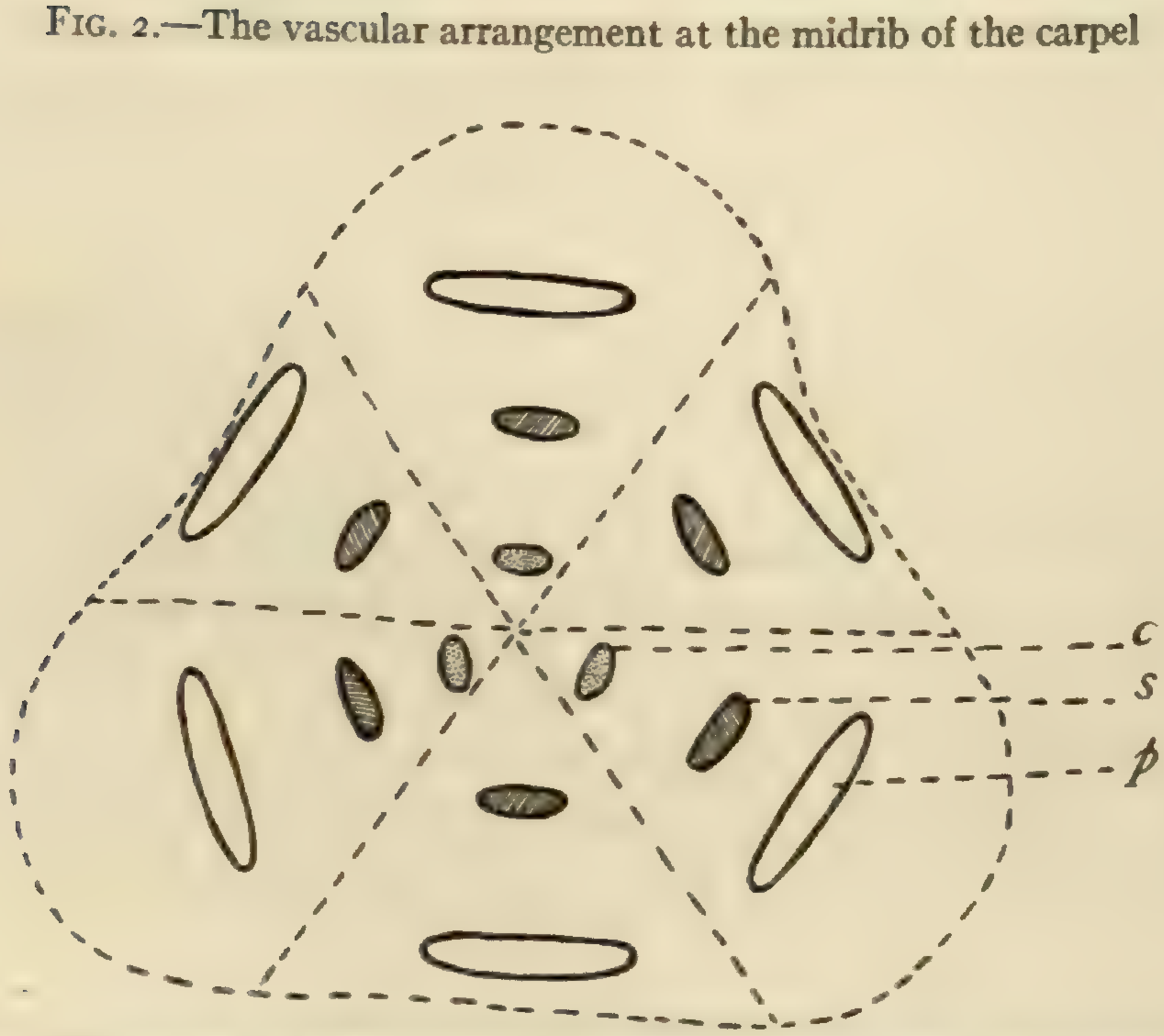

FIG. 3.—Diagram of a flower of *Cyrtanthus* against the ovary (dotted line), showing the relation between the vascular conditions and the parts of the flower; *p*, petals; *s*, sepals; *c*, carpels.

antipodals, synergids, and egg showed no departures from this type in the mature stage of the gametophyte; the development up to this stage was not seen.

Embryo

The literature of the embryos of monocotyledons is extensive but not satisfactory. The "sheath" was recognized, of course, early in the history of the study, and was presumed to be a single cotyledon, giving the name to the whole class. Besides the presence of the single cotyledon, early investigators were so impressed with the large amount of endosperm or "albumen," as they called it, occurring outside the embryos in such seeds, that they also called them "albuminous." As to the origin and nature of the cotyledonary sheath, various opinions are expressed, which fact perhaps demonstrates that there are various modes of origin. The earliest writers, HANSTEIN (3) and FAMINTZIN (4), described the embryo of *Alisma Plantago*, in which the cotyledon is terminal in origin. HEGELMAIER (5) describes it in some cases as arising from a cell near the tip, and being pushed into the terminal position by later development. SHAFFNER (6) says that in *Sagittaria* the cotyledon arises from the terminal cell of the proembryo. CAMPBELL (7) finds the same condition in *Naias;* the same author in his study of *Lilaea* (8) says that the sheath is not at first an enveloping organ, but that it becomes such by the lateral growth of its margins; and the same facts are repeated for the Araceae (9). WITTMACK (10) in his monograph on the Bromeliaceae has a drawing of a longitudinal section of the embryo of *Guzmannia tricolor*, taken through the center in such a way as to show the elongated side of the sheath on one side and the shorter side on the other. He calls the long side the "scutellum" and the short side the "cotyledon." There is nothing in his figure to show that scutellum and cotyledon are not one and the same structure, and yet it would seem that so reliable an investigator must have had some reason for applying the two terms in this way. BILLINGS (11) says that the cotyledon is terminal in origin, that the middle segment of the three-celled proembryo gives rise to all the other organs, and that before the stem tip is differentiated, the cells surrounding the area where it is to arise grow up into a ridge of tissue, which in the mature embryo incloses the growing point completely. Here the author seems to imply (1) that the sheath does not completely inclose the growing point in its inception, and (2) that the sheath is not the cotyledon, in the latter respect agreeing with WITTMACK.

A new impulse was given to the study of embryology when COULTER and CHAMBERLAIN began the revision of their *Morphology of gymnosperms* (15). Especially was the impulse felt among the cycads. Cycads were collected from the oriental and occidental tropics, and all phases of their life history investigated. The dicotyledonous nature of the cycadean embryo was demonstrated for all of them, even that of *Ceratozamia* (12, 13), which had been reported as having a single cotyledon. But while these embryos were shown to be normally dicotyledonous, exceptions to dicotyledony were seen to be by no means rare. The case of *Ceratozamia* was proved to be the result of abortion; but in *Microcycas* a condition was found in which the cotyledons were fused to such an extent that the author of the investigation referred to the fused structure as a "sheath," and expressed the suspicion that monocotyledony, even in angiosperms, might have arisen in both these ways: suppression, as in *Ceratozamia*, and fusion, as in *Microcycas*. COULTER and CHAMBERLAIN, in their chapter on evolutionary tendencies (15), seem to give credence to this suspicion by requesting Sister HELEN ANGELA to illustrate her views on the subject of the primitiveness of polycotyledony and the tendency to reduce the number of cotyledons.

From the material at my disposal, I was able to procure embryos in two different stages of development. The younger one was found to consist of an enveloping sheath, still meristematic and with four distinct lobes at its apex. Each lobe has its own vascular strand, four separate strands arising from the four poles of the root. The lobes are approximately equal in size at this stage, but not absolutely so, as can be seen from fig. 2. At the base of the sheath is the region from which the stem tip will arise later; at this stage it is not meristematic. Figs. 1 and 2 of the plate are sketches of the exterior and interior of the embryo at this stage, and fig. 3 is a cross-section which illustrates the irregular growth in thickness of the sheath, the region which bears the vascular strands resisting the pressure from without and giving the appearance of lobes and a four-sided aperture.

The second embryo studied (fig. 4) is older than the one just described. The sheath now consists of two regions, a lower portion

which still envelops the growing point, and a long upper projection which has resulted from the greater growth in length of one side of the sheath. The tip of the shorter, aborted side of the sheath is seen at *a* in figs. 4, 5, and 15. The vacant space in this older embryo, unlike that of the younger, which was four-lobed, is now reduced to the narrow slit represented by *s* in figs. 13 and 14. The vascular conditions are indicated in fig. 5. In the lower region of the sheath (the completely enveloping region below the abortion) each side has two vascular strands, making four in all (figs. 8–13), which arise independently from the cotyledonary node and enter the two differentiated sides of the sheath, just as happens in many dicotyledonous seedlings (figs. 5 and 7). Near the tip of the lower or aborted side of the sheath, the vascular strands from that side abruptly enter the region of the extended side, and fuse with the vascular strands of that organ.

At this stage of development, the growing point has differentiated the first and second leaves, the first arising on the side corresponding to the aborted side of the sheath, and the second one about opposite the first. All are closely pressed upon by the growing sides of the sheath. These arrangements are shown in figs. 5 and 10. The root cylinder is tetrarch (fig. 6).

Discussion

As was remarked before, the amount of endosperm in the seeds of *Cyrtanthus* is very great in proportion to the size of the embryo. This makes it difficult for the embryo to develop its organs to their full extent. I have noted how the originally large space within the sides of the sheath is reduced, little by little, as growth progresses, to a very small space (*s*, figs. 3, 13, and 14). Following out this process in thought, it is not difficult to imagine how the early condition indicated in fig. 3 might easily be changed into that of figs. 4 and 5 merely by the mechanical pressure of the large endosperm. In other words, the sheath of monocotyledons is probably a fusion of two or more cotyledons; the probability amounting almost to a certainty when we remember that the very same condition here described in a monocotyledon has been discovered in the dicotyledonous embryo of *Microcycas*, a complete fusion of the

two cotyledons. Looking at fig. 1 of pl. V in the *Microcycas* paper (14), and comparing it with my fig. 13, one would be puzzled to say which is the dicotyledon and which the monocotyledon. The same difficulty would arise by comparing my fig. 10 with fig. 13 of pl. VI of the *Microcycas* paper. Sister HELEN ANGELA was so impressed with the complete fusion of the two cotyledons that she called the fused structure a sheath.

Furthermore, a consideration of the vascular connections, the four root poles with their extensions finding full outlet in the sheath, shows that this sheath represents the whole cotyledonary apparatus, which, historically, finds its expression in many cotyledons in *Pinus*, in two in the normal cycads and dicotyledonous angiosperms, and in the sheath of monocotyledons.

This condition seems to me almost the last proof necessary to demonstrate the origin of monocotyledons from dicotyledons.

Summary

1. The embryo sac of *Cyrtanthus* seems to follow the regular *Lilium* type. The endosperm is very extensive.

2. Stomata are more numerous on the inner than on the outer surface of the carpel.

3. There are three separate bundles at the midrib of each carpel and two at the fusion of the carpels. This arrangement is related to the various parts of the flower.

4. The youngest observed stages of the embryo have the stem tip enveloped by a sheath with four lobes at its top.

5. In an older embryo the sheath is differentiated into a longer and a shorter side, the appearance and vascular anatomy of which give the distinct impression of two cotyledons.

6. Any pressure or fusion is referred to the extraordinary amount of endosperm.

7. The investigation is considered a last proof of the theory of monocotyledony from dicotyledony.

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EXPLANATION OF PLATE XXIV

The drawings were made with the aid of an Abbé camera lucida, the magnification used being 120 diameters. In every case, *a* indicates the tip of the aborted or short side of the sheath, *c* the longer side, and *s* the space within the sheath.

FIG. 1.—Exterior view of a young embryo before the differentiation of the two portions of the sheath.

FIG. 2.—Longitudinal section through the center of the embryo shown in fig. 1.

FIG. 3.—Cross-section of a young sheath above the region of the growing point of the stem.

FIG. 4.—Exterior view of an older embryo, showing differentiation of both sides of the sheath.

FIG. 5.—Longitudinal section (partly reconstructed and diagrammatic), showing the first leaf, the growing point, and the behavior of the vascular bundles.

FIG. 6.—Cross-section through the root cylinder of the older embryo.

FIG. 7.—Cross-section of the cotyledonary node, showing the independent origin of the four bundles.

FIG. 8.—Cross-section just above the cotyledonary plate; the four bundles have assumed the vertical position.

FIG. 9.—Cross-section above that represented in fig. 8, showing the stem cylinder.

FIG. 10.—Section above the preceding, showing the first leaf and the growing point.

FIG. 11.—Cross-section above the tip of the second leaf.

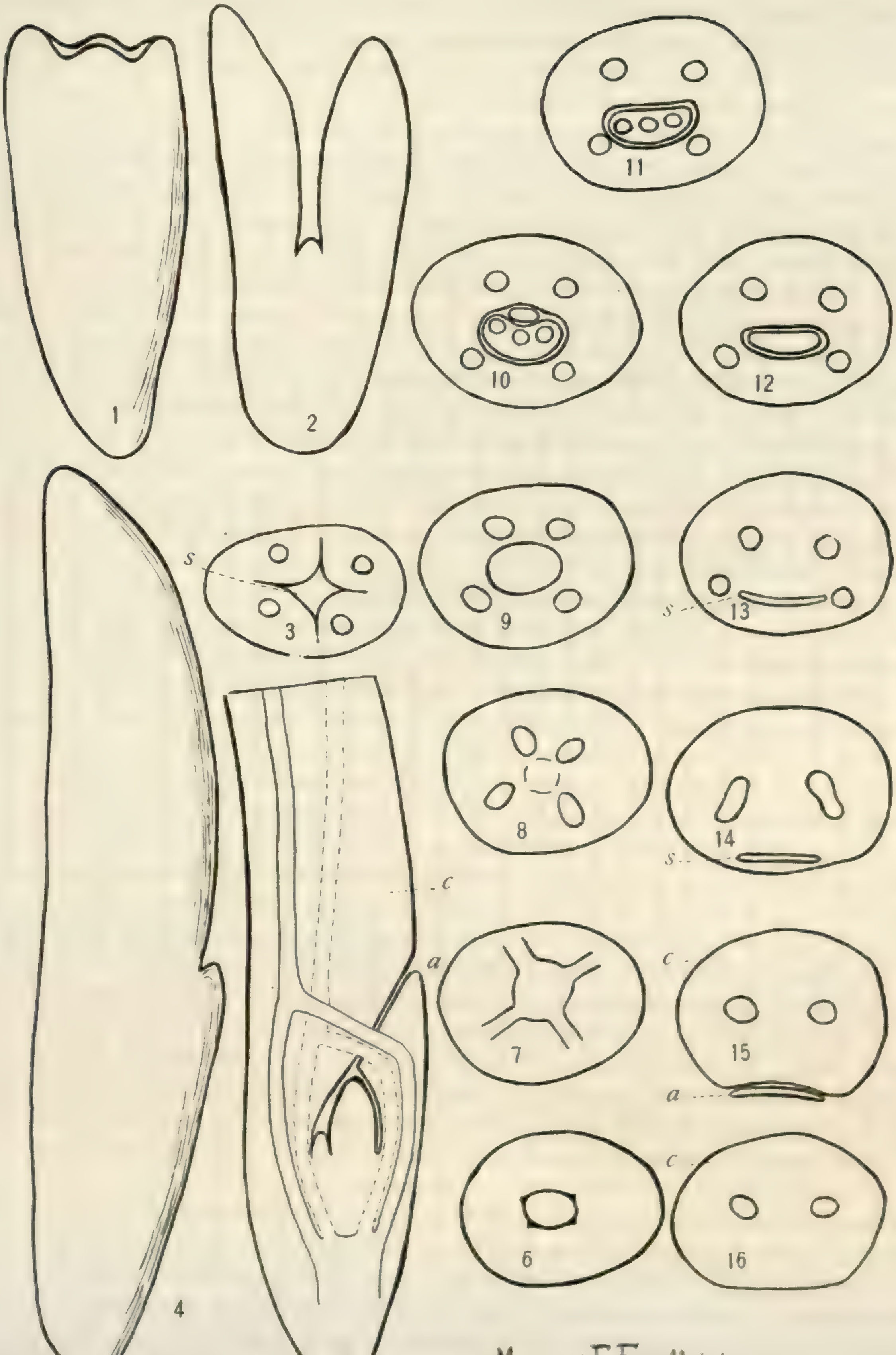
FIG. 12.—Cross-section just above that shown in fig. 11; it shows the tip of the first leaf.

FIG. 13.—Cross-section above the tip of the first leaf; shows the space or slit between the two sides of the sheath.

FIG. 14.—A section showing the fusion of the four bundles to make two; it was made immediately above that shown in fig. 13.

FIG. 15.—A section near the tip of the aborted side of the sheath, giving the appearance of two cotyledons lying side by side.

FIG. 16.—A cross-section in the upper part of the sheath.



Margaret E. Farrell del.

FARRELL on CYRTANTHUS

CURRENT LITERATURE

BOOK REVIEWS

Textile fibers

A book by STIRM[1] on the chemical technology of textile fibers deals with every phase of the derivation and handling of the fibers: source, method of obtaining, physical and chemical character, washing and bleaching of the raw fiber, preparation for stamping and dyeing, and stamping and dyeing themselves. The work covers plant fibers (cotton and various bast fibers, such as flax, hemp, jute, and manila), animal fibers (wool, hair, and silk), mineral fibers (asbestos), and artificially produced fibers from mineral raw materials (glass and mineral fiber) and from plant raw materials (India rubber fiber and artificial silk). The work on plant fibers reminds the botanist of the detail with which these technical workers have studied the histology, chemistry, and physics of cotton, bast, etc. In the case of cotton, one finds a good summary of our knowledge of the histology and microchemistry of the fiber. The chemical composition of the fiber is given, including the substances involved as impurities, with methods of removal. The chemistry of cellulose is dealt with briefly, with various theories as to its molecular weight and structural formula. The behavior of cellulose toward many reagents in various concentrations (acids, bases, salts, oxidizing and hydrolizing agents, and various solvents) is clearly summarized. The changes involved in such processes as bleaching, mercerization, and formation of artificial silk are discussed, along with the theories offered in explanation. The author recognizes many of the problems with cellulose as belonging to the field of colloidal chemistry. The histology and chemistry of bast fibers receive like consideration. The work reminds one that the knowledge useful to the technical worker is often the same as that which is of significance to the "pure" scientist.—WILLIAM CROCKER.

MINOR NOTICES

The Journal of Ecology.—It is rare that a new journal starts off so well as does the *Journal of Ecology*,[2] the first volume of which is now completed. In 1904 a great advance was made in ecological study in the British Isles by the

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[1] STIRM, KARL, Chemische Technologie der Gespinnstfasern. xvi+410. Berlin: Gebrüder Borntraeger. 1913.

[2] The Journal of Ecology, edited for the British Ecological Society by FRANK CAVERS. Cambridge University Press (American agent: The University of Chicago Press). Volume I. 1913. Price per annum, $3.75.

organization of the Central Committee for the Survey and Study of British Vegetation, more popularly known as the British Vegetation Committee. This organization secured the effective cooperation of British ecologists, and among other things has to its credit the organization of the International Phytogeographic Excursion of 1911 and the publication of *Types of British vegetation*. In 1912 there was formally organized the British Ecological Society, which has become the successor of the British Vegetation Committee. One of the chief tasks of this new society is the publication of a journal. The first thought was to have this new journal be little more than an organ of the British ecologists. But a broader view prevailed, and we now have an international journal devoted to ecology, the first of its kind; from the first number the quality of the journal has been such as to give it rank with the foremost botanical periodicals.

The scope of the new ecological journal is such as to make an effective appeal to ecologists of other lands quite as much as to those of the British Isles. To facilitate use by both general and local readers, articles and reviews of general nature are kept distinct from those of more local significance. To the writer the "high-water mark" of the new journal seems to be reached by its reviews. It is doubtful if the reviews in any other botanical journal equal in efficiency those of the new *Journal of Ecology*. They are models as to thoroughness and accuracy, in many cases making it really unnecessary to consult the original papers except to secure details. It is unusual and highly illuminating to have published in many of the reviews the more significant illustrations of the original papers. The only adverse criticism that the writer would make is that the reviews generally are unsigned. It goes without saying that the new journal is absolutely necessary for the working ecologist. The British ecologists may well congratulate themselves upon their accomplishments during the last decade. In 1904 their work was scattered and mostly of local significance; today they are unquestionably the leaders in cooperative ecological activity.—H. C. COWLES.

American Journal of Botany.—The first number of this new journal has appeared, and needs no explanation to American botanists. It is the official publication of the Botanical Society of America, and is published by the Brooklyn Botanic Garden. The introductory statement by the editor-in-chief, Professor F. C. NEWCOMBE of the University of Michigan, shows that the increase of means of publication has not kept pace with production. "The result has been that our established botanical journals in America are overstocked with manuscripts waiting their turn, our colleges and universities are making outlets for their own production, and foreign journals have their courtesy and capacity taxed by the offers of American contributors. All three of the conditions just named are undesirable: an author does not like to wait a year or more for the publication of his paper, the multiplication of small periodicals by colleges and universities is a vexation to research, and it is neither

just to ourselves nor kind to our colleagues of other lands to ask them to give large printing space to our contributions." It is evident that the new journal will relieve this pressure somewhat, which the established journals have felt keenly.

This initial number contains the following papers: "The development of *Agaricus arvensis* and *A. comtulus*," by GEO. F. ATKINSON; "Studies of teratological phenomena in their relation to evolution and the problems of heredity. I," by ORLAND E. WHITE; "Nuclear behavior in the promycelia of *Caeoma nitens* Burrill and *Puccinia Peckiana* Howe," by L. O. KUNKEL; and "An axial abscission of *Impatiens Sultani* as the result of traumatic stimuli," by R. A. GORTNER and J. A. HARRIS.—J. M. C.

Illinois Academy of Science.—The volume of *Transactions* of the Illinois Academy of Science for 1913 contains the following botanical papers: "Annotated list of the algae of eastern Illinois," by E. N. TRANSEAU; "Reproduction by layering in the black spruce," by GEO. D. FULLER; "Evaporation and soil moisture on the prairies of Illinois," by E. M. HARVEY; and "The stratification of atmospheric humidity in the forest," by GEO. D. FULLER, J. R. LOCKE, and WADE MCNUTT.—J. M. C.

NOTES FOR STUDENTS

Paleobotanical notes.—ARBER[3] has done a most useful service in revising the seed-impressions of the British Coal Measures, and putting them into more definite categories. The most recent list, that of KIDSTON in 1894, included 5 genera with 19 species. ARBER'S revision contains 14 genera with 37 species. These detached seed-impressions belong to both Cycadofilicales and Cordaitales, whose seeds cannot be distinguished. Of the 14 genera recognized 9 are new (*Platyspermum*, *Cornucarpus*, *Samarospermum*, *Microspermum*, *Megalospermum*, *Radiospermum*, *Neurospermum*, *Schizospermum*, *Pterospermum*). In addition to the diagnosis of genera and species, every British species is figured.

Mrs. ARBER[4] has examined sections of a new specimen of *Trigonocarpus*, showing that the sclerenchyma of the micropylar beak is preserved as far as its extreme apex, and also that the nucellus was free from the integument almost to the base of the seed.

KNOWLTON[5] has described a collection of Jurassic plants from Alaska, obtained between latitudes 68° and 69°. SEWARD'S report on a collection of

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[3] ARBER, E. A. NEWELL, A revision of the seed-impressions of the British Coal Measures. Ann. Botany **28**:81–108. *figs. 8. pls. 6–8.* 1914.

[4] ARBER, AGNES, A note on *Trigonocarpus*. Ann. Botany **28**:195, 196. *fig. 1.* 1914.

[5] KNOWLTON, F. H., The Jurassic flora of Cape Lisburne, Alaska. U.S. Geol. Survey. Professional paper 85-D. pp. 39–55. *pls. 5–8.* 1914.

Jurassic plants from Siberia (Amurland) makes a comparison of Alaskan and Siberian Jurassic floras possible. KNOWLTON concludes that the striking similarity between the Jurassic floras of northwestern North America and eastern Siberia shows that the land connection between these regions during the Jurassic must have been practically continuous. The Alaskan list recognizes 12 genera and 17 species, the solitary species of *Equisetum* being described as new. The dominance of cycadophytes is obvious, so far as it can be inferred from so small a number of species, the list being as follows: pteridophytes 5; cycadophytes 11; and *Ginkgo* represented by a single species.

SALISBURY[6] has described in detail a new species of *Trigonocarpus* obtained from the Lower Coal Measures at Shore Littleborough, England. In addition to the well marked features of the sclerotesta and sarcotesta, the nucellus is almost completely free from the integument, and has a well developed and thick walled epidermal layer, three longitudinal flanges corresponding with the commissures, and numerous secretory sacs in the ground tissue. The conclusion is reached that this species is more primitive than its congeners, and that the testa arose from the lateral fusion of a whorl of six originally free members. A discussion of the resemblances and differences between the Trigonocarpeae and the Lagenostomales reaches the conclusion that they are to be explained by intercalated growth, followed by subsequent fusion of the nucellus and integument.

Dr. STOPES[7] has published a very interesting lecture upon the past and future of paleobotany, delivered at University College, London. The thesis is that paleobotany is now an independent science, contributing to both botany and geology, and with its own important field.

THOMAS and BANCROFT[8] have made a detailed comparative study of the cuticle of recent and fossil cycads, including also mention of other gymnosperms, especially with reference to the form and structure of the stomata. The general conclusion is that the characters presented by the stomata and epidermal cells of gymnosperms are important as indicating to some extent their relationships. Among the cycadophytes there are some characters which have undergone little modification from the Jurassic to the present time, and the authors conclude that stomatal structure is the expression of ancestral characters rather than of purely local and temporary conditions of environment.—J. M. C.

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[6] SALISBURY, E. J., On the structure and relationships of *Trigonocarpus shorensis*, sp. nov. A new seed from the Paleozoic rocks. Ann. Botany **28**:39–80. *figs. 8. pls. 4, 5.* 1914.

[7] STOPES, MARIE C., Paleobotany; its past and its future. Knowledge **37**: 15–24. 1914.

[8] THOMAS, H. HAMSHAW, and BANCROFT, NELLIE, On the cuticles of some recent and fossil cycadean fronds. Trans. Linn. Soc. London II. Bot. **8**:155–204. *figs. 32. pls. 17–20.* 1913.

Hydrogen and alcoholic fermentation.—Through a study of the reduction of methylene blue, in the presence of yeast, LVOFF[9] has found further evidence that reductase plays an essential rôle in alcoholic fermentation. Methylene blue is rapidly decolorized when placed in a yeast culture. This decoloration is brought about through the absorption of two atoms of nascent hydrogen (molecular H is not effective) at the double bond of the color group. Reductase activates the hydrogen. He finds the output of alcohol and CO_2 greatly lowered while the methylene blue is being reduced. Therefore the hydrogen, activated by the reductase, probably goes directly to the methylene blue molecule, thereby arresting the further normal steps of the fermentation process. Quantitative determinations of the CO_2 and alcohol produced and the hydrogen absorbed (by the methylene blue) showed that one gram-molecule of methylene blue takes from the fermentation medium one gram-molecule of hydrogen and "inactivates" one gram-molecule of hexose, thus preventing the splitting into alcohol and CO_2. Unfortunately, a study of this "inactivated" carbohydrate molecule has not been made.

Again, yeast when mixed with water only still has the capacity for reducing methylene blue. CO_2 is given off at the same time in amount directly related to the methylene blue reduced; for example, one gram-molecule methylene blue, under conditions favoring self-fermentation, takes one gram-molecule of hydrogen from the medium, liberating one gram-molecule of CO_2. The source of this CO_2 is yet unexplained. However, the author suggests the possibility that it comes from the fermentation of amino acids in the yeast, a suggestion in agreement with the work of EHRLICH, and particularly with that of BACH, where from amino acids in the presence of alloxan, NH_3, CO_2, and 2H are eliminated (the 2H passing to the alloxan), leaving an aldehyde in the medium. —F. M. HARVEY.

Cytology and embryology of Smilacina.—*Smilacina* was studied some time ago by LAWSON,[10] who reported that synapsis is due not to a marked contraction of the nuclear contents, but to a sudden enlargement of the nuclear cavity, which gives the appearance of a contraction. MCALLISTER[11] claims that synapsis is due to contraction and not to any considerable enlargement of the nuclear cavity. It would seem as if this should be settled by measurement rather than by discussion, but since both men studied *Smilacina* and both made measurements, an extensive series of measurements of various forms would seem to be in order.

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[9] LVOFF, GERGIUS, Hefegärung und Wasserstoff. Zeitschr. Gärungsphysiol. **3**: 289–320. 1914.

[10] LAWSON, A. A., The phase of the nucleus known as synapsis. Trans. Roy. Soc. Edinburgh **47**:591–604. *pls. 2.* 1911. Rev. in BOT. GAZ. **51**:313. 1911.

[11] MCALLISTER, F., On the cytology and embryology of *Smilacina racemosa*. Trans. Wis. Acad. Sci. **17**:599–660. *pls. 56–58.* 1913.

During synapsis McALLISTER finds that there is a lateral pairing and fusion of spirems, the fusion being complete at the time of the recovery from synapsis. After this recovery there is a second contraction stage. The double heterotypic chromosomes are formed, not by the approximation of the limbs of loops, but by a transverse segmentation of a longitudinally split spirem, the line of the split probably representing the line of approximation of the two parental spirems seen at synapsis.

The heterotypic and homotypic mitoses in the megaspore mother cell result in the formation of four megaspores, separated by plasma membranes. The membrane formed at the heterotypic mitosis persists, while those formed at the homotypic mitosis quickly break down. From the inner binucleate cell, thus formed, an 8-nucleate embryo sac is developed. Consequently, two megaspores are concerned in the development of the embryo sac.

Adventitious embryos develop from nucellar cells in the micropylar region, and one or more of them may become mature. The presence of pollen tubes indicates that embryos may also result from fertilization.—CHARLES J. CHAMBERLAIN.

Marine algae.—BORGESEN[12] has published an account of the marine Chlorophyceae of the Danish West Indies, based upon collections made in 1892, 1895, and 1905. The list contains 34 genera including 86 species, 4 new species being described in *Cladophora* (2), *Avrainvillea*, and *Pringsheimia*. The full field notes and the numerous illustrations make the account an exceedingly satisfactory one.

BORGENSEN[13] has also given an account of the species of *Sargassum* collected during his three visits to the Danish West Indies, partly along the coasts of the islands, and partly in the Sargasso Sea. The shore collections include 4 species, while the pelagic collections are all referred to two species. The discussion of the "biology, affinities, and origin of the gulfweed" is especially interesting. The conclusions reached are that the gulfweed of the Sargasso Sea consists of two species, *S. natans* (most common) and *S. Hystrix*, var. *fluitans;* that they are true pelagic algae, living perennially on the open sea; and that most probably they have descended from shore forms of the West Indies and the neighboring American coast. The author says "it is of great interest that we have an instance of floating, pelagic species of such a high alga type as *Sargassum;* because, as is well known, the higher types of algae are as a rule attached, and if detached they perish sooner or later."—J. M. C.

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[12] BORGENSEN, F., The marine algae of the Danish West Indies. Part I. Chlorophyceae. Dansk Botanisk Arkiv 1: no. 4. pp. 158. *figs. 126* and *chart*. 1913. Copenhagen: H. Hagerup. *Kr* 4.

[13] ———, The species of *Sargassum* found along the coasts of the Danish West Indies, with remarks upon the floating forms of the Sargasso Sea. Mindeskrift for Japetus Steenstrup. pp. 20. *figs. 8*. 1914.

Reproduction in Scenedesmus.—Although *Scenedesmus* is a very familiar alga, the details of its reproduction had not been investigated, doubtless because the cells are so small. SMITH[14] has studied the cell structure and reproduction in all of the three species. The cells are strictly uninucleate, contain a single pyrenoid, and have a cell wall consisting of two layers. When 4-celled colonies are formed, the steps are as follows: The first nuclear division is followed by a transverse cleavage of the cytoplasm, and nuclear division in the two resulting protoplasts is followed by cleavage furrows at right angles to the first furrow. The pyrenoid then disappears and the uninucleate protoplasts elongate until they extend the entire length of the mother cell. A pyrenoid is now formed *de novo* within each of the four cells, and cell walls appear. The young colony escapes through a longitudinal rupture in the wall of the mother cell and assumes the characteristic arrangement by unrolling. In the formation of 8-celled colonies, there are three nuclear divisions, the second and third being followed by cytoplasmic cleavages. The material for the study was grown in pure cultures. Sections were cut from 3–5 μ in thickness and stained in Flemming's safranin-gentian violet-orange combination.—C. J. CHAMBERLAIN.

The embryo of Gyrostachys.—The origin and development of the embryo sac of *Gyrostachys*, more commonly known as *Spiranthes*, is described for two species, *S. gracilis* and *S. cernua*, by Miss PACE,[15] whose previous work allows her to speak with authority upon this subject. The embryo sac is very irregular in its development, sometimes arising from 4 megaspores, sometimes from 2, and sometimes from only one. At the fertilization stage the embryo sac may contain 4, 5, 6, or 8 nuclei, the 6-nucleate condition, resulting from a lack of one mitosis in the chalazal end of the sac, being the most frequent. The diploid number of chromosomes in *S. gracilis* is 30, and in *S. cernua* 60; consequently, the relation, in this respect, is similar to that between *Oenothera Lamarckiana* and *O. gigas*, and *S. cernua* might be called a tetraploid form. As is well known, *S. cernua* is a larger and more vigorous species, and the gigantism is evident also in the size of the ovary, the ovules, and the size of the cells. Miss PACE suggests that the subject might be worth investigating experimentally.—CHARLES J. CHAMBERLAIN.

Westphalian Calamariaceae.—A revision of the European Calamariaceae, undertaken in conjunction with KIDSTON, impressed upon JONGMANS the fragmentary condition of our knowledge of the Calamariceae of the Rheinish-Westphalian coal fields, and also the inadequacy of the descriptions and figures. Accordingly, with the cooperation of KUKUK,[16] he examined and described

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[14] SMITH, GILBERT M., The cell structure and colony formation in *Scenedesmus*. Archiv für Protistenkunde **32**:278–297. *pls. 16, 17.* 1914.

[15] PACE, LULA, Two species of *Gyrostachys*. Baylor University Bull. **17**:1–16. *pl. 1.* 1914.

[16] JONGMANS, W. J., and KUKUK, P., Die Calamariaceen des Rheinisch-Westfälischen Kohlenbeckens. Mededeelingen van's Rikjs Herbarium. Leiden. no. 20. Text 8vo. pp. 89. Atlas 4to. *pls. 22.* 1913.

specimens from the following collections: K. Pr. Geol. Landesanstalt, Berlin; Städt. Museum, Chemnitz; Naturw. Museum, Dortmund; K. K. Geol. Reichsanstalt, Wien; Märkisches Museum, Witten; Museum, Osnabrück; s'Rijks Herbarium, Leiden; Geol. Institut der Universität, Göttingen; Geol. Institut der Bergakademie, Clausthal; and Geol. Institut der Universität, Münster i.W.

The work is strictly taxonomic. The descriptions and synonomy are very complete, and the magnificent plates enable one to study the subject almost as well as if he had the actual specimens in his hands.—CHARLES J. CHAMBERLAIN.

Anatomy of petioles of Cycads.—LE GOC[17] has studied the debated xylem situation in the foliar bundles of the cycads. As is well known, both centripetal and centrifugal xylem occur, a situation which has been variously interpreted, but in general the bundle has been spoken of as mesarch. LE GOC has found that at the very base of the petioles in cycads the xylem is entirely centrifugal, and that later the bundles assume different forms, becoming concentric, collateral, or a combination of these two arrangements. He regards the centrifugal xylem at the base as a secondary growth, and the centripetal xylem which appears later as a primary structure, laid down early, but only gradually lignified. The two xylems he thinks are probably distinct in origin, appearing in response to "physiological demands but morphologically discontinuous." During most of the course of the bundles along the petiole the two xylems remain distinct, and therefore he would suggest that the bundle is more properly called "pseudo-mesarch."—J. M. C.

The origin of the "eye spot."—From a study of the literature, without any apparent study of the structures themselves, ROTHERT[18] comes to the conclusion that the "eye spot" of flagellates and algae is derived from a plastid. The evidence seemed clear already, so far as the Euglenaceae are concerned, and GUIGNARD, as long ago as 1889, claimed that the "eye spot" seen in the sperms of *Fucus* arose from a colorless plastid. ROTHERT overlooks entirely the various papers by YAMANOUCHI from 1909 to 1913, particularly the paper on *Cutleria*,[19] in which the origin and development of the "eye spot" are described in detail.—C. J. CHAMBERLAIN.

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[17] LE GOC, M. J., Observations on the centripetal and centrifugal xylems in the petioles of cycads. Ann. Botany **28**:183–193. *pl. 11. fig. 1.* 1914.

[18] ROTHERT, WLADISLAW, Der "Augenfleck" der Algen und Flagellaten ein Chromoplast. Ber Deutsch. Bot. Gesells. **32**:91–96. 1914.

[19] YAMANOUCHI, SHIGÉO, The life history of *Cutleria*. BOT. GAZ. **54**:441–502. *figs. 16. pls. 26–45.* 1912.

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THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

JUNE 1914

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JUNE 1914

WINTER AS A FACTOR IN THE XEROPHILY OF CERTAIN EVERGREEN ERICADS[1]

FRANK CALEB GATES

(WITH TWELVE FIGURES)

Of late years considerable attention has been drawn toward the apparently anomalous condition of several plants with obvious xerophytic modifications living in bogs with an apparently unlimited water supply. Many explanations of this apparent anomaly have been attempted. It was with a desire to obtain further knowledge upon the question that the author entered upon this piece of research work in the Botanical Department of the University of Michigan in the fall of 1910.

The work was carried on under the direction and supervision of Professor F. C. NEWCOMBE. To him I am greatly indebted both for the opportunity to work and for his stimulating criticism throughout the work. To Dr. H. A. GLEASON and to Dr. J. B. POLLOCK, both of the University of Michigan, I am also indebted for helpful conferences during the course of the work. To Mr. W. B. MCDOUGALL, of the University of Michigan, I am further indebted for the examination of material for the presence of mycorhiza. The nomenclature is that of the seventh edition of GRAY'S *Manual*.

[1] Contribution from the Botanical Laboratory of the University of Michigan, no. 136. The part of this work done during the summer of 1912 was carried on with the aid of a grant from the American Association for the Advancement of Science.

General discussion

In order to maintain existence, it is necessary for an organism to fulfil the fundamental requirements of life; it must be able to take in food; it must be able to digest its food; it must be able to oxidize or otherwise rearrange its substance to obtain energy; it must be able to eliminate its waste products; and it must be able to perpetuate its kind. Further, it must be able to perform all these functions in its particular, individual environment. As these individual plants cannot migrate, they must be able to accommodate themselves to the changing environmental conditions or die. That they flourish from year to year in healthy condition is unquestionable evidence that they are able to cope with their environment. Their ability to invade genetically lower associations of plants indicates that they are thriving rather than just merely existing in their habitat.

Although a living plant is always the expression of the integration of environmental and hereditary factors, the most important single factor in the environment is the physiological water supply. The modifications of plant structure which lead to the conservation of the water supply are termed *xerophytic adaptations* or *xerophytic reactions*. The presence of xerophytic adaptations does not necessarily predicate that the amount of water used by the plant is relatively small, but that the ratio of the amount used to that which the plant obtains tends to become less than unity. Some so-called xerophytic plants use as much or more than ordinary mesophytic plants, as GROOM (**20**) found was the case with *Larix decidua*. They are xerophytic, however, because they cannot absorb a large amount of water in proportion to that which they could otherwise transpire.

This is particularly true in the summer, when plants have their transpiring organs. The loss of leaves during the winter is quite rightly regarded as a xerophytic adaptation. The bog ericads which were investigated, however, retain their leaves during the winter. This opens at once the question, are these plants xerophytes because of their summer or their winter environment? As it may be safely assumed that the evergreen habit is hereditary in these ericads, the reaction to the environment necessitates the xerophily.

It would seem, at first glance, that plants which grow in bogs, where there is an obvious physical water supply, would not be restricted in its use, but the various xerophytic adaptations argue for the conservation of water in the plant. This fact led investigators to ask why the plants could not make full use of the water present. Many answers have been attempted, and it seems quite likely that the true answer is a combination of the different reasons rather than any one. The problem presents an obvious result obtained from a bewildering mass of causes, whose interactions are not yet known.

The ability of peat bog plants to absorb water is limited on account of poorly developed, shallow root systems (FRÜH and SCHRÖTER 18), low oxygen content of the water (DACHNOWSKI 9 and HESSELMANN 23), low aeration (TRANSEAU 49, DACHNOWSKI 10, and FREE 17), root excretions (LIVINGSTON, BRITTON, and REID 27, and SCHREINER and REED 45, 46), bog toxins (LIVINGSTON 29 and DACHNOWSKI 9, 10, 11), the necessity of mycorhizal fungi in some species, the low temperature of the soil water (KOSAROFF 25, FRÜH and SCHRÖTER 18, and especially TRANSEAU 49), and biological processes rather than chemical differences in the soil (DACHNOWSKI 12). Much stress cannot be laid upon the acidity of the soil, as has been done by SCHIMPER (44), because of the findings of later investigations. The acidity is very low and differs in different bog associations (TRANSEAU 49). That acidity is a necessary factor in the soil for the growth of trailing arbutus (*Epigaea repens*) and of the blueberry (*Vaccinium corymbosum*) was most admirably demonstrated by COVILLE (6, 7), who found that poor aeration was usually the real cause of poor growth and not acidity. Acidity, however, may be inimical to certain crops. SAMPSON and ALLEN (42) found that, as a rule, some of the common acids accelerate transpiration, and that weak solutions often produce as marked effects as strong ones.

The water absorbed is conducted up through the stems. A study of stem structure would show whether the ericads differ essentially from other bog shrubs. In either case the ability to conduct water must be adequate, as the plants thrive. From the stem the water passes into the leaves, where the largest

part of it is vaporized and passes out of the plant through the stomates.

Xerophytic responses may be classified into means retarding transpiration in the leaves or transpiring organs, absorption in the roots, checking transportation in the conducting tissue, or provision for an accumulation of water. In the peat bog ericads used during the course of this investigation, xerophytic response is very evident in the leaves, but is not accompanied by water storage tissue, which makes the xerophytic structure more necessary on account of the poorly developed root sytem. The evergreen habit, with its relatively large exposure of leaf surface, calls for greater activity of the root system throughout the winter, for transpiration still continues even when the thermometer is below zero. That means to reduce the loss of water are all the more necessary under winter conditions is obvious.

Seasonal history of peat bog plants

EVERGREEN ERICADS

During the winter the leaves of all of the evergreen ericads, *Chamaedaphne calyculata*, *Andromeda glaucophylla*, and *Vaccinium macrocarpon*, are upright, a position in which they receive a minimum of direct sunlight. The leaves are dark red or brown in color. With the coming of spring the old leaves curve outward or downward, resulting in an increase of the direct sunlight which they receive. At the same time the leaves become dark green in color. The season's growth of young leaves takes place soon after flowering. At first the young leaves are upright, but in a short time they bend outward. As soon as the young leaves are fully developed, the old leaves gradually drop off. In the case of *Vaccinium macrocarpon*, however, some of the leaves may be retained for two or three years. With the coming of the next winter, the leaves of these plants gradually bend up into an upright position and their color changes from bright green through dark green to shades of red and brown. The color changes begin at the margins of the leaves and work toward the midribs. In a mild winter the basal portion of midribs of *Chamaedaphne* may remain green the entire winter. *Vaccinium* and *Andromeda* are usually protected by a covering of

snow, but they exhibit these changes of position and color irrespective of that fact.

DECIDUOUS TREES AND SHRUBS

The principal trees are *Larix laricina* and *Acer rubrum*, and the commonest shrubs are *Aronia melanocarpa*, *Salix pedicellaris*, *S. discolor*, *S. sericea*, *Spiraea salicifolia*, *Betula pumila*, *Nemopanthes mucronata*, *Ilex verticillata*, *Gaylussacia baccata*, *Cornus paniculata*, *Cephalanthus occidentalis*, *Sambucus canadensis*, and *Rosa carolina*.

During the winter, bog trees and shrubs are leafless, which greatly reduces the transpiration. The snow that is present during the winter protects the root system and lower part of the stem from danger from excessively low temperature, but the upper parts of the trees and shrubs are not so protected. They must be able to resist water loss through their own modification. This is sufficient for the severest winters in southern Michigan. During the winter of 1911–1912, *Spiraea* was the only deciduous shrub to be killed down to the snow line.

With the opening of spring the buds swell and develop into branches bearing leaves which carry on the work of the season. A separation layer is formed upon the approach of winter at the base of the petiole, and by the time winter has set in the leaves have fallen.

HERBACEOUS PLANTS

The seasonal history of the herbaceous bog plants follows two general lines: the plant which has developed during the growing season may die down completely before winter, leaving seeds to reproduce it the following year, or it may die down to the ground and be vegetatively reproduced the following year from underground stems, bulbs, rootstocks, or buds. Any of these ways is an absolute xerophytic adaptation on account of winter conditions, but does not interfere with summer development.

Structure of certain peat bog plants

ROOT SYSTEM

Without exception, all of the forms dealt with had a very shallow root system, which was usually very poorly developed. It is in direct contrast to that of the xerophytes of the desert (CANNON 4).

Two general types of roots could be separated, according to the presence or absence of mycorhizal fungi. Most ericads have mycorhiza, but none were found upon the roots of *Andromeda* or *Chamaedaphne*. In working over the material furnished him by the author, McDOUGALL found that *Andromeda* occasionally gave evidences of mycorhizal appearance, although further investigation failed to reveal its presence. Mycorhiza was found on *Larix laricina*, *Acer rubrum*, and *Vaccinium macrocarpon*, but was not noticed on any of the following plants: *Carex filiformis*, *Sagittaria latifolia*, *Eupatorium perfoliatum*, *Dulichium arundinaceum*, *Asclepias incarnata*, and *Aspidium thelypteris*.

The absence of mycorhiza on *Chamaedaphne* and these other plants demonstrates that it is not a necessary adaptation to the bog environment. The presence of resin deposits (TRANSEAU **49**) is often a noteworthy feature of the roots of bog plants. Root hairs were not observed, although TRANSEAU (**49**) found that in culture solutions, which were well aerated, normal roots with root hairs were produced in *Larix*.

During the summer, the roots of the bog plants, at least apparently, have an abundant water supply, although as a matter of fact the *Sphagnum* which surrounds the roots may be physiologically dry, even when apparently wet, on account of its great ability to soak up and retain water (FRÜH and SCHRÖTER **18**, and DAVIS **14**). In general, however, there is standing water beyond the ability of the *Sphagnum* to absorb, and therefore the bog plants have a supply to draw on throughout a normal season. Seasons of drought, therefore, would be the critical ones, and that of 1911 was a case in hand. In so far as could be observed, it did not appear that the ericads were suffering from lack of water even on the hottest and driest days. The leathery nature of their leaves makes it nearly impossible to tell whether the plants are wilting or not, even when herbaceous vegetation was obviously wilted. In the natural distribution of these plants, droughts are not sufficiently extreme nor of sufficient duration to dry out the *Sphagnum*. The great ability of *Sphagnum* to soak up and retain water localizes the water within reach at the expense of the surrounding area. During the summer this often results in the elevation of the water table

under the bog several inches above that of the surrounding country.

Taking into consideration the rarity of real drought conditions of long duration, it is evident that the root system in the bog habitat is able more than merely to maintain these plants within their normal range throughout drought conditions. The xerophytic adaptations of the transpiring organs, of course, materially aid by lessening the demand upon root absorption.

During the winter the ground is normally frozen. On account of the low position of bogs, they are more subject to early and late freezes than the surrounding country. Although the ground may be frozen, the covering of snow prevents the access of very low temperatures to the roots. In spite of the fact that the ground is frozen, it is evident from the continual water loss of the above-ground parts that some water is being absorbed by the roots, quite likely the water vapor evaporated from the ice into the spaces which become opened around the roots soon after the freezing of the ground.

At any of the temperatures at which roots were dug up (down to -10° C.) it did not appear that any part of the plant was frozen. All parts were pliable to handling. The exposure of severed parts of *Chamaedaphne* for one-half an hour to -25° C. resulted in freezing and loss of pliability. It was repeatedly noticed that the leaves which had been exposed to the severest weather of the winter, including a temperature of -29° C., were dry, and cracked when bent. Later it became evident that these leaves had been killed. Beyond this simple test, whose limits of accuracy are not known, there were no suitable means of determining in the field whether the plant tissue was frozen.

CONDUCTING SYSTEM

The conducting system in bog ericads consists of a very narrow ring of young xylem just outside the old wood (fig. 1). There is a very striking similarity in the appearance of the cross-section of the three ericads studied. The type of stem represented in bog ericads is strikingly different from that of other bog shrubs in the relatively smaller amount of conducting tissue and in the smaller

lumina of its cells. In this respect bog herbs are all different from the bog ericads also. Among the bog shrubs the ericad type stands out distinctly from all the other shrubs, there being far less difference between the structure of any two ericads than between an ericad and any other bog plant.

Just how the water is conducted from the roots to the transpiring organs is not a closed question. For a discussion of it the reader is referred to the literature, particularly COPELAND (5), DIXON (16), OVERTON (37), RENNER (41), SCHERMBEEK (43), and BABCOCK (1). The results of this investigation show that the fundamental control of rate of conduction is exercised by transpiration. An increase of transpiration always means an increase of conduction, and a decrease in transpiration means a decrease in conduction, though not always in the same proportion. Absorption and conduction are more closely related to each other than to transpiration, and they are more closely related to the turgidity of the cells than is transpiration. By reducing the turgidity of the cells transpiration exercises a control over the other two.

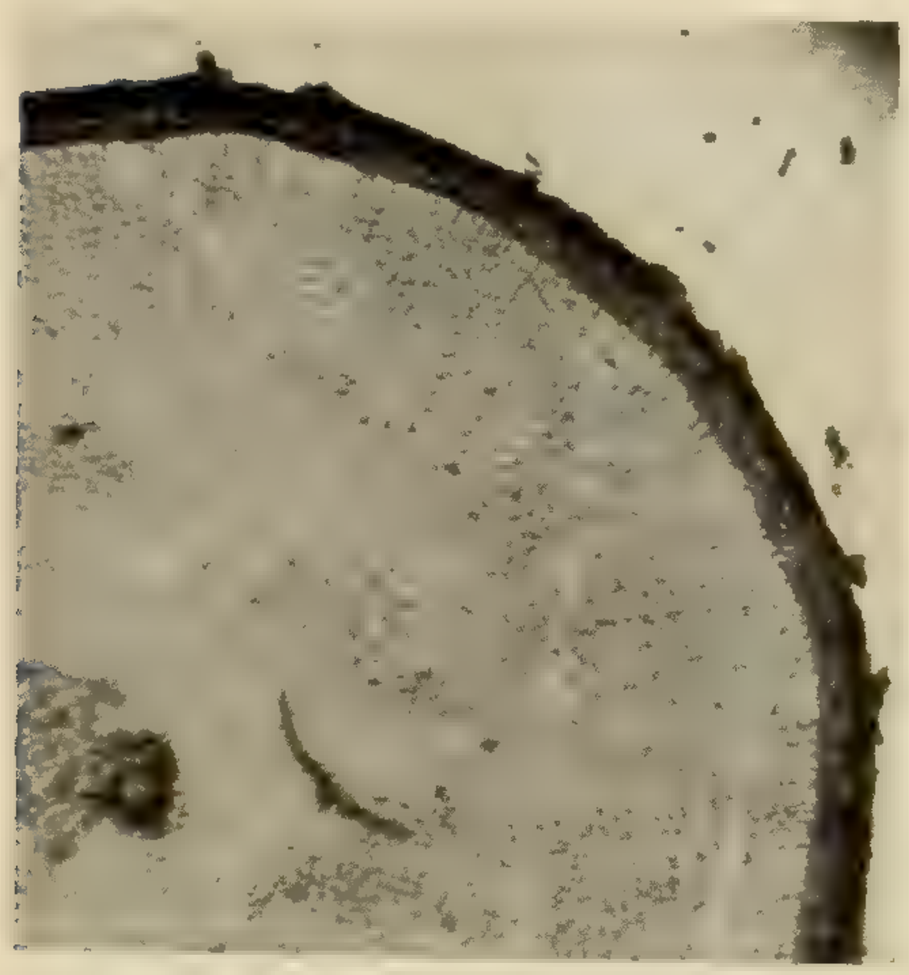


FIG. 1.—Photomicrograph of the stem of *Chamaedaphne calyculata:* the dark outer ring is cortex, just within it is the medium colored young xylem, then the light colored wood, and in the center the pith.

UTILIZATION SYSTEM

Although the external appearance of the leaves of various peat bog plants is very different, the general internal structure is more nearly similar, and that of the various ericads is still more alike. Several well marked xerophytic adaptations are present, notably the strongly cuticularized epidermis, absence of stomates on the upper surface, a well developed palisade layer one to three cells thick, frequently sunken stomates, and coatings of wax, bloom, hairs, or scales. Mechanical tissue is present and accounts for the suppression of the ordinary symptoms of wilting. Usually the

leaves are at least slightly revolute, those of *Andromeda* and *Salix candida* strongly so. The leaves are usually dark green in color, but often reddish at the beginning and close of the vegetative season. The abundant presence of cutin in the evergreen ericads as an efficient xerophytic adaptation against loss of water at all times, but especially in winter, has been brought out by WIEGAND (**51**).

A considerable amount of water is transpired by many bog plants, and the loss may be as great as or greater than that from mesophytic plants of the same vicinity. This suggests that it is the maximum rate to which the plant may be subjected rather than the amount of water lost that is the important consideration. If the amount of food material is correlated with the amount of water lost, there would have to be considerably more water absorbed in the bog habitat, as it is notably deficient in available mineral food material (TRANSEAU **49**). Plants that normally grow in non-bog conditions, as white pine and black spruce, when growing under bog conditions are much dwarfed and stunted, and their leaves exhibit very pronounced xerophytic modifications, so much so that these plants growing in bogs have received specific designation.

Some plants, as *Populus tremuloides* and *Poa pratensis*, that may grow in either bog or mesophytic soil, do better in the latter situation and always exhibit a pronounced xerophily in the bog soil. Some plants demand bog conditions and even then have xerophytic modifications, as COVILLE (**6**) demonstrated in the case of the blueberry.

During the growing season, all of the bog plants have their transpiring organs, but the great majority do not retain them during the winter. In every case where leaves are retained, their winter position is different from their summer one. The winter position is usually upright, but in the evergreen conifers the leaves are more closely appressed to the twig. The young leaf as it comes out in the spring is also upright and remains so at least as long as it is tender. The fact that the dark upper surface is innermost when the leaves are upright serves to protect it by reducing the amount of radiant energy absorbed, which would raise the temperature of the mesophyll cells and lead to greater loss of water. The under surface is already well protected. The upright, upper surface to upper

surface position of the leaves during winter is really a xerophytic modification, reducing the amount of radiant energy absorbed at a time when it would be needlessly dissipated in increase of water loss, which the absence of phytosynthesis and the closure of the stomates does not occasion. Leaves which develop upon *Chamaedaphne* in the *Larix* association are much less xerophytically modified. They are much more subject to winter killing.

The loss of water by the leaves exercises a twofold function. The excess of radiant energy absorbed and not used in photosynthesis could easily raise the temperature of the leaf to the death point during hot waves, were it not dissipated in vaporizing water. DARWIN (13), through the use of a resistance thermometer, demonstrated that, with the check to transpiration that comes with induced closure of the stomates, the temperature of the leaf rises. Normally this higher temperature would not occur, for the excess of radiant energy being used to vaporize water causes a lowering of temperature. The loss of water in the leaves maintains a stream of water from the roots up. This is necessary for the removal of the products of respiration (BABCOCK 1) and for the lifting of the absorbed mineral material to the leaves. Water is also necessary in photosynthesis.

As LIVINGSTON (30) puts it: "The total amount of transpirational water lost from a plant, for any given period, may be considered as a summation of the effects of the evaporating power of the air and of the radiant energy absorbed throughout the period, modified by certain secondary effects of these conditions and certain responses to other conditions." The ratio of the water income to that of the removal must not fall below unity for any considerable time in plants which are not water-storing. Quoting again from LIVINGSTON (31): "The really crucial question with regard to any soil . . . . is . . . . *at what rate, and for how long a time, can it deliver water to a unit area of a water-absorbing surface?*" That water is supplied in sufficient quantities during the most extreme conditions of summer that obtain in nature in this region is evident from this investigation. The opposite statement is true for winter, namely, that in very severe winters the removal of water from the exposed parts of certain plants is so in excess of the supply that too

thorough drying and therefore death result. This same process has already been shown by KIHLMAN (**24**) to be the cause of the arctic tree line.

That the water supply for ericads in Michigan peat bogs is actually ample to their needs is clearly demonstrated by experimentation upon potted plants, for even under the very extreme evaporating power of the air on July 5, 1912, the maximum rate of transpiration was contemporaneous with the maximum evaporating power of the air. Conditions of atmospheric evaporating power in Michigan are never as high as those of Arizona, where LLOYD (**32**) found that the fall in the rate of transpiration in ocotillo (*Fouquieria splendens*) occurred before that of the maximum evaporating power of the air. Whether these results may be the true expression of the behavior of rooted plants may be open to question, as LLOYD used cuttings to experiment with. It was found in the present investigation that on days of extreme evaporating power in Michigan a decline in the transpiration rate in advance of the time of maximum evaporating power of the air did actually occur in cuttings, but was not exhibited in potted plants of the same species. Such a check in transpiration is occasioned by what LIVINGSTON and BROWN (**28**) have termed "incipient drying," in the course of which the evaporating menisci have retreated into the pores of the cells, thereby not only decreasing the amount of the exposed surfaces, but also greatly increasing the surface tension of these evaporating surfaces, which decreases the vapor tension and consequently the rate of vaporization (RENNER **39, 40,** and PATTEN **38**). The increase in the concentration of cell sap which accompanies this check in water removal further retards vaporization. A very serviceable pictorial presentation of the matter is given by MACDOUGAL (**34**).

The recent work of some investigators seems to withdraw the foundations from the theory of the efficient function of the stomates as the regulators of transpiration (LLOYD **32, 33**, and others). That closed stomates are efficient means of lowering transpiration has been demonstrated by many authors (BURGERSTEIN **3**, and DELF **15**). The closure of the stomates of evergreen plants during winter, which has been demonstrated by several investigators,

especially STAHL (47), is an important factor in reducing transpiration at that season, when the water intake is at best very low. The work of LLOYD (32) on *Fouquieria* led him to conclude that the capacity of the diffusion of the stomates was well in excess of what would be required for the greatest observed transpiration rate. F. DARWIN (DELF 15), in a preliminary account before the British Association, concluded that if the stomates can be observed by a sufficiently delicate method, the stomal movements will be found to correspond closely with changes in the rate of transpiration caused by alteration in external conditions.

In the present investigation, in which the method of relative time of penetration of an oil was used to indicate the condition of the stomates, there was no evidence that the stomates exercised a "closely regulatory" function. The stomates opened in the morning, in general in the diffused light of dawn, but the rate of transpiration showed no sudden rise, but rather kept proportional to that of the evaporating power of the air. In the afternoon the stomates did not begin to close until after the beginning of the decline in transpiration. This was true both in potted plants and in cuttings properly cared for on days that were not extreme. In many cuttings the closure of the stomates seemed to be due to the shock of cutting rather than to any excessive water loss. Almost all of the wilted plants had their stomates closed, but in dried leaves the stomates were open.

The experimentation on plants in the field led to the conclusion that the stomates were open during the hours of sunshine, and that, although the opening of the stomates preceded the rise in transpiration in the morning, the decline in transpiration set in in the afternoon before the beginning of closing of the stomates. The rate of transpiration sank more quickly to a lower level than the time it took the stomates to close could possibly account for.

Experimentation

MATERIALS AND METHODS[2]

Throughout the study of this problem an experimental method was used which yielded numerical data. The experiments were carried on upon bog plants, principally *Chamaedaphne calyculata*

[2] Cf. BURGERSTEIN 3.

(L.) Moench, obtained from First Sister Lake, a little west of Ann Arbor, Michigan, and at Mud Lake in the northern part of Washtenaw County, Michigan.

Toward the close of autumn in 1910 and 1911, plants of the evergreen ericads were potted and kept outdoors under prevailing conditions. During the middle of winter their transpiration was determined by successive weighings on a beam balance sensitive to 0.002 gram. The pot was inclosed by an aluminum shell (devised by GANONG) closed at the top with rubber dam and sealed with wax, whereby the water loss was limited to the plants, as controls repeatedly demonstrated. Readings of weight to 0.01 gram, temperature by mercury thermometer and thermograph, relative humidity by wet and dry bulb thermometers, and general conditions of the weather were recorded.

A number of pottings of ericads and other plants were made at First Sister Lake, June 1, 1912, the plants allowed to develop under bog conditions, and experimented upon the first week of July 1912.

By far the greater part of the work, however, was conducted with cuttings. These were made from the plants at First Sister Lake, immediately cut under water in jars and brought into the laboratory where they were again cut under water. The cuttings were then set up in two-holed rubber corks in bottles of distilled water and sealed with vaseline. A thermometer inserted in the other hole of the cork gave the temperature of the water. Work with controls proved that the apparatus was water-vapor tight. Usually about one-half an hour was allowed for adjustment before measurements were commenced. Weighings were made at intervals of one, two, or more hours, according to the purpose of the experiment. Such experiments were seldom carried over 24 hours, except for special reasons. The day of 24 hours of 100 "minutes" each was used in recording experiments, because of its obvious convenience.

Some plants which were transplanted into the greenhouse in *Sphagnum* did so poorly that no experiments were made upon them. Shoots that developed on cut twigs kept in water in the laboratory or greenhouse seldom lived more than a couple of weeks, and as their internal structure was not normal, no experiments were performed upon them.

It was found that different *Chamaedaphne* plants from the *Chamaedaphne* association (fig. 2) transpired at virtually the same rate during the same experiment. Consequently, that plant was taken as the basis for all comparisons and was included in practically every set of experiments.

With the close of the experiment the leaves were detached, placed side by side on white paper, covered with a thin piece of glass, and their outlines traced with a polar planimeter, by which means the leaf area was obtained. After a little practice with this instrument it was found that successive determinations of the same set of leaves did not vary by as much as 0.3 per cent. Accordingly the average of two determinations was used throughout the work. When stomates were also present on the upper surfaces (the rare exception in the plants used), the area thus obtained was doubled.

With the data so recorded, the results of each experiment were calculated with the aid of a slide rule to a standard basis, the rate of transpiration in grams per hour per 100 sq. cm. of leaf surface. These results were plotted on cross-section paper and the comparison made. As more than one determination for each plant was made, the resulting curves should approach a general similarity. Under the same conditions the similarity of the graphs was striking. Through dissimilarity of the resulting graphs, it was possible both to demonstrate the effect of change of experimental conditions and to weed out aberrant plants. As the greater part of the work was concerned with relative values, the continued reappearance of the same result in the graphs was taken to uphold the contention and no contention not thus uniformly upheld is presented in this paper.

During a part of the year 1912 the volume of the leaves was also determined by ascertaining the amount of alcohol they displaced. Alcohol was used in place of water on account of the large amount of air coating the leaves submerged in the latter. The results were calculated on the basis of water loss per hour per 1 cc. of volume. This was done in order to incorporate the results obtained from plants, the difficulty of determining the leaf surface of which would otherwise have rendered it virtually impossible. The correlation

of volume and leaf surface was irregular. With leaves of about the same size and thickness, the amount of water loss varied proportionally. In plants of *Chamaedaphne* from different plant associations, where both size and thickness of the leaves varied (fig. 2), it did not usually appear that volume was any constant function of the leaf area.

While it is recognized that the measured leaf area is not the area of the water-losing mesophyll cells, it is believed that it furnishes

FIG. 2.—Twigs of *Chamaedaphne calyculata*, showing the character of the leaves developed in the *Larix* association (bottles 1 and 2) and in the *Chamaedaphne* association (bottle 3), at First Sister Lake; April 29, 1911.

a satisfactory basis of comparison attained without the excessive difficulty that would attend the determination of the actual area of the surface abutting upon the intercellular spaces. The leaf surface, moreover, is the area through which the diffusion into the outer air takes place.

To obtain a knowledge of the evaporating power of the air an open dish of water was run with several of the experiments.

At the close of many of the experiments a section of the smallest part of the stem below any transpiring organs was cut out,

preserved in glycerin-alcohol, later sectioned, and stained with iodin green to show the area of the conducting system.

By means of the lithium nitrate method, which consists of cutting off the lower ends of leafy stems under a 0.5 per cent aqueous solution of $Li(NO_3)_2$, allowing the stems to take up the solution, removing after certain intervals, and cutting immediately into 1 cm. lengths, testing these pieces in the spectroscope for the presence of lithium, a knowledge of the rate of conduction under different conditions was obtained. This method only approximates the rate of conduction in rooted plants, which could not be used because of inability to know when the lithium nitrate would be absorbed by the roots.

The difficulty of examining the stomates of *Chamaedaphne* by the ordinary method of stripping and the consequent uncertainty of its results with this species led to the abandonment of work on stomates until the publication of a new method (MOLISCH **35**) opened the way for experimentation upon this pertinent question. The "infiltration method," as it is called, depends upon the fact that when a leaf is wetted with a penetrating liquid, such as absolute alcohol, xylol, or turpentine, and held up to the light, it becomes translucent as soon as the liquid has penetrated the leaf. The relative time that it takes the leaf to become translucent after the application of xylol indicates whether the stomates are open or closed, because the more the stomates are open, the easier and quicker will the liquid penetrate the tissue and the sooner will it become translucent. A "normal" time must be determined for each species upon which to base deviations. Xylol was used throughout the present work, and the results were checked up with absolute alcohol and turpentine. As the method is so very simple, it can be employed in the field and several determinations made each time. The results were remarkably uniform.

EXPERIMENTATION DURING THE WINTER

Transpiration

During winter the transpiration of the plants of the region is reduced to a serviceable minimum which, however, is not zero (cf. KUSANO **26**). For herbaceous plants the minimum is lower

than for shrubs and trees. No experimentation was performed upon herbaceous plants during the winter, for it is known how exceedingly small is the amount of water loss from seeds, and as the vegetative means of reproduction employed by other herbs are

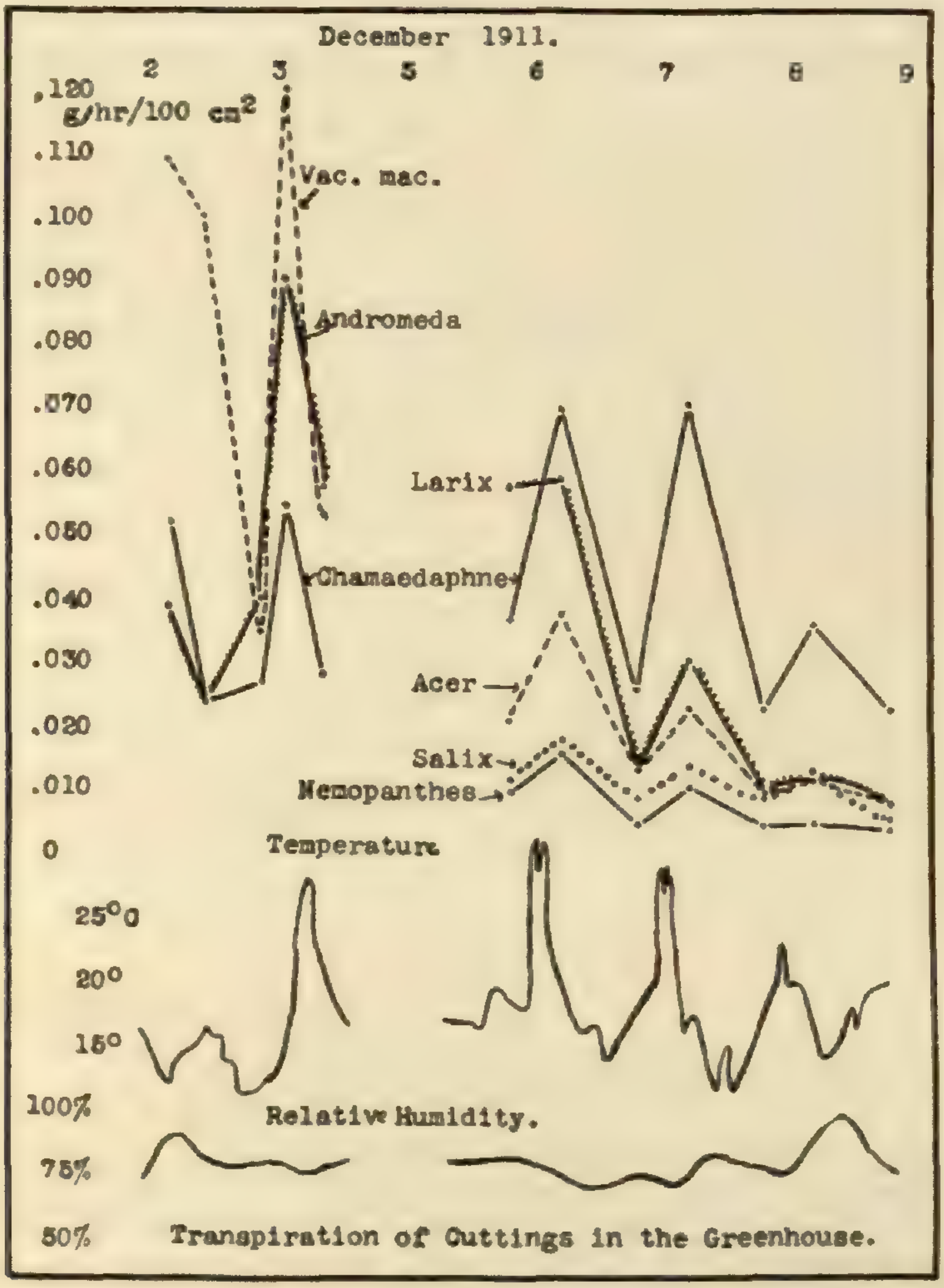


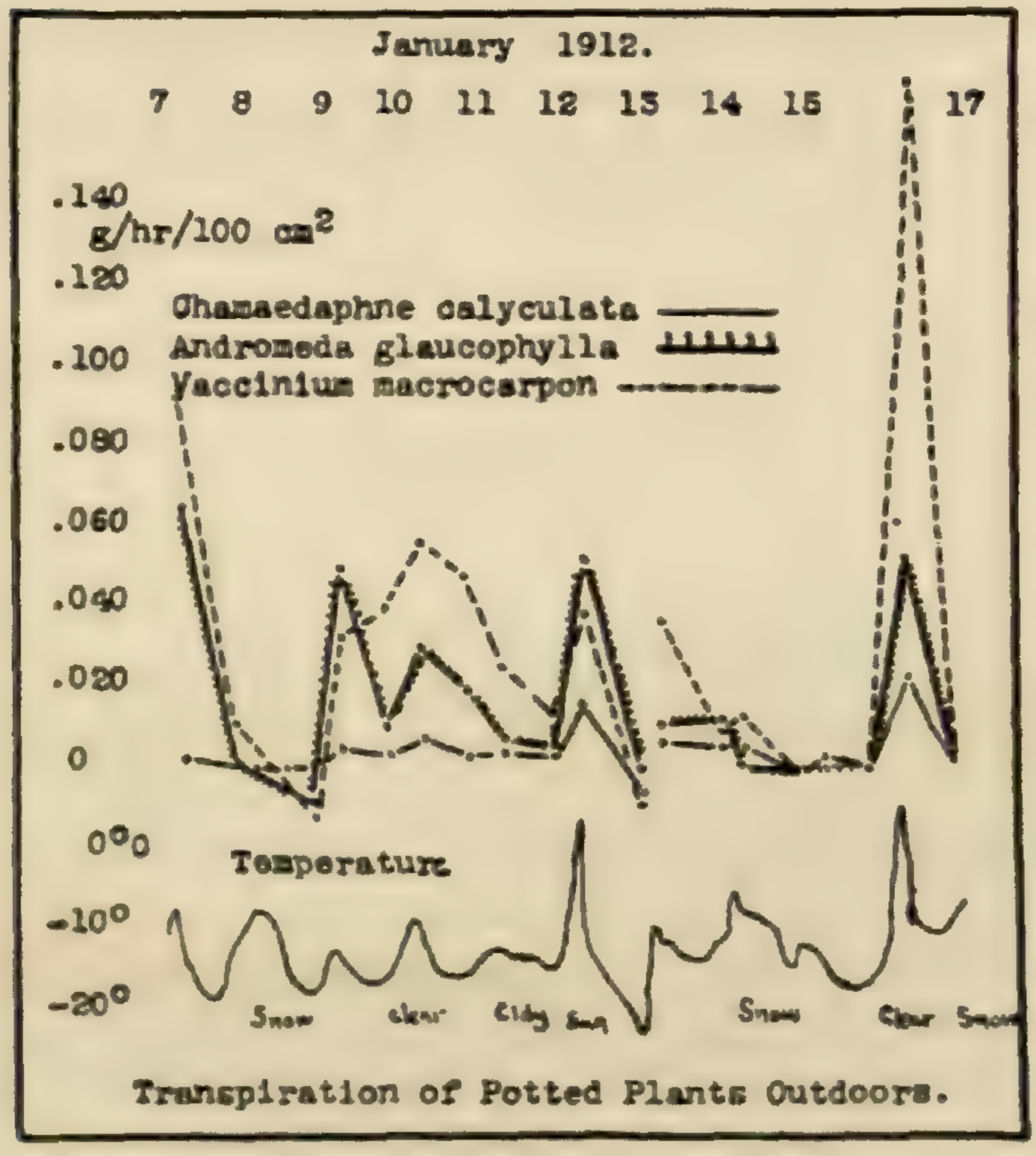
FIG. 3.—Transpiration of cuttings of *Acer rubrum*, *Andromeda glaucophylla*, *Chamaedaphne calyculata*, *Larix laricina*, *Nemopanthes mucronata*, *Salix pedicellaris*, and *Vaccinium macrocarpon* in the greenhouse.

underground and thoroughly protected from exposure, no comparison could be made with the ericads which retain their plant body subject to constant exposure throughout the winter.

The purpose of the winter experimentation, therefore, was to obtain a knowledge of the transpiration of several of the shrubs and trees, and compare that of the leaf-retaining ericads with that of the deciduous shrubs, under winter conditions outdoors and under

laboratory conditions which simulated the severest conditions which could obtain in nature during the winter.

Experimentation was carried on both with potted plants and with cuttings indoors and outdoors. A few of the graphs obtained from the data from these experiments are given in figs. 3–6. Although they have been selected from the general array of data to avoid needless repetition they represent the general conclusions, not merely special cases.



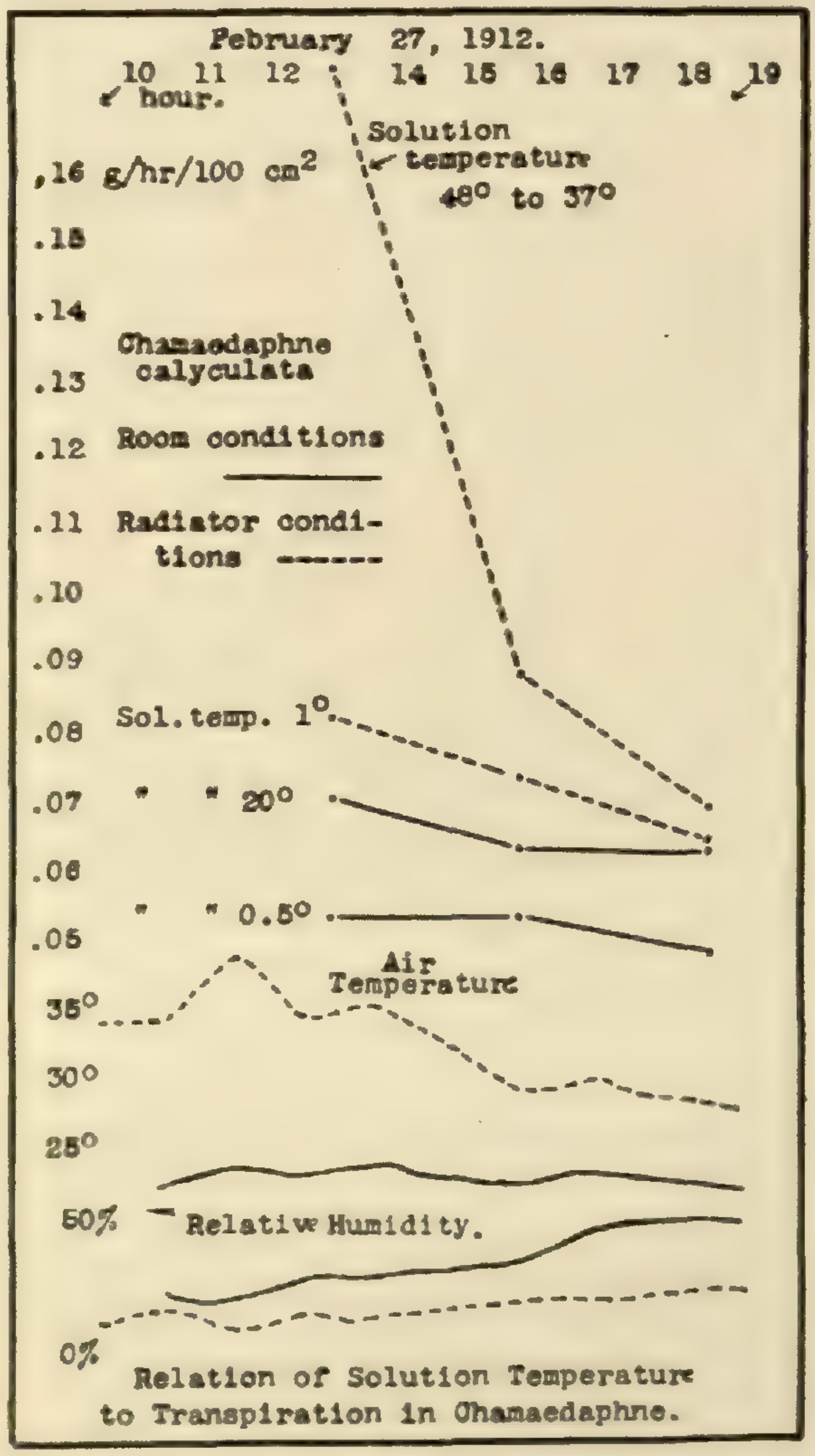
Transpiration of Potted Plants Outdoors.

FIG. 4.—Transpiration of potted plants of *Andromeda glaucophylla*, *Chamaedaphne calyculata*, and *Vaccinium macrocarpon* outdoors in winter.

Consideration of these data clearly indicates that the transpiration of these bog plants is very low in winter. Furthermore, with scarcely an exception, the rate of water loss is much greater (2–15 times) in the evergreen ericads than in the leafless shrubs and trees. When the very much more exposed position of the deciduous trees and most of the deciduous shrubs is taken into account, the difference in the rate of transpiration in nature is accentuated. The mere position of the ericads near the ground serves to reduce water loss. This same relation holds among the ericads themselves, namely, that the greater the rate of transpiration under given conditions, the more protected is the position in which that species grows. For example, *Chamaedaphne* transpires at a lower rate than *Andromeda* and *Vaccinium;* and *Chamaedaphne*, because of its higher growth, is more exposed. YAPP (52) has shown that the nearer the ground in a closed association the lower the evaporating power of the air.

These data support the well known facts that transpiration

varies directly with the temperature, inversely with the relative humidity, and is greater in daylight than in darkness. The last may be almost entirely included in the two former, as the absorption of radiant energy during the day would increase the temperature of the leaf were it not used up in augmenting transpiration. For example, on February 27–29, with a temperature constant to within 2° and the relative humidity constant to within 5 per cent, in plants of *Chamaedaphne* which were run in the laboratory with light about 0.1 per cent of sunlight, not at any time being exposed to direct sunlight, the rate of transpiration was very noticeably higher (0.071 gm. and 0.083 gm.) during the diffuse daylight than in the periods of darkness before and after. During the night following, with the temperature constant but the humidity dropping slightly, the transpiration of these same duplicates decreased to 0.046 gm. and 0.060 gm. respectively.



Relation of Solution Temperature to Transpiration in Chamaedaphne.

FIG. 5.—The relation of solution temperature to transpiration in *Chamaedaphne calyculata*.

Only rarely does the rate of transpiration exceed 0.01 gm./hr./100 sq. cm. in any of the plants experimented with under winter conditions. With the exception of *Vaccinium macrocarpon*, the rate was more often less than 0.005 gm. than above it. In the deciduous shrubs it was usually below 0.001 gm., and not infrequently was hardly within the power of measurement with

the means employed. The advantage of the deciduous habit in reducing transpiration in the winter is apparent.

Per unit area of leafless twig surface, bog shrubs lose less water than do the bog trees under the conditions of these experiments. Furthermore, bog shrubs are more subject to winter killing in a severe winter in this latitude unless protected by snow. This would seem to indicate that the xerophytism of the bog trees was in general more efficient than that of the bog shrubs under extreme winter conditions, or that their means of water renewal was more efficient.

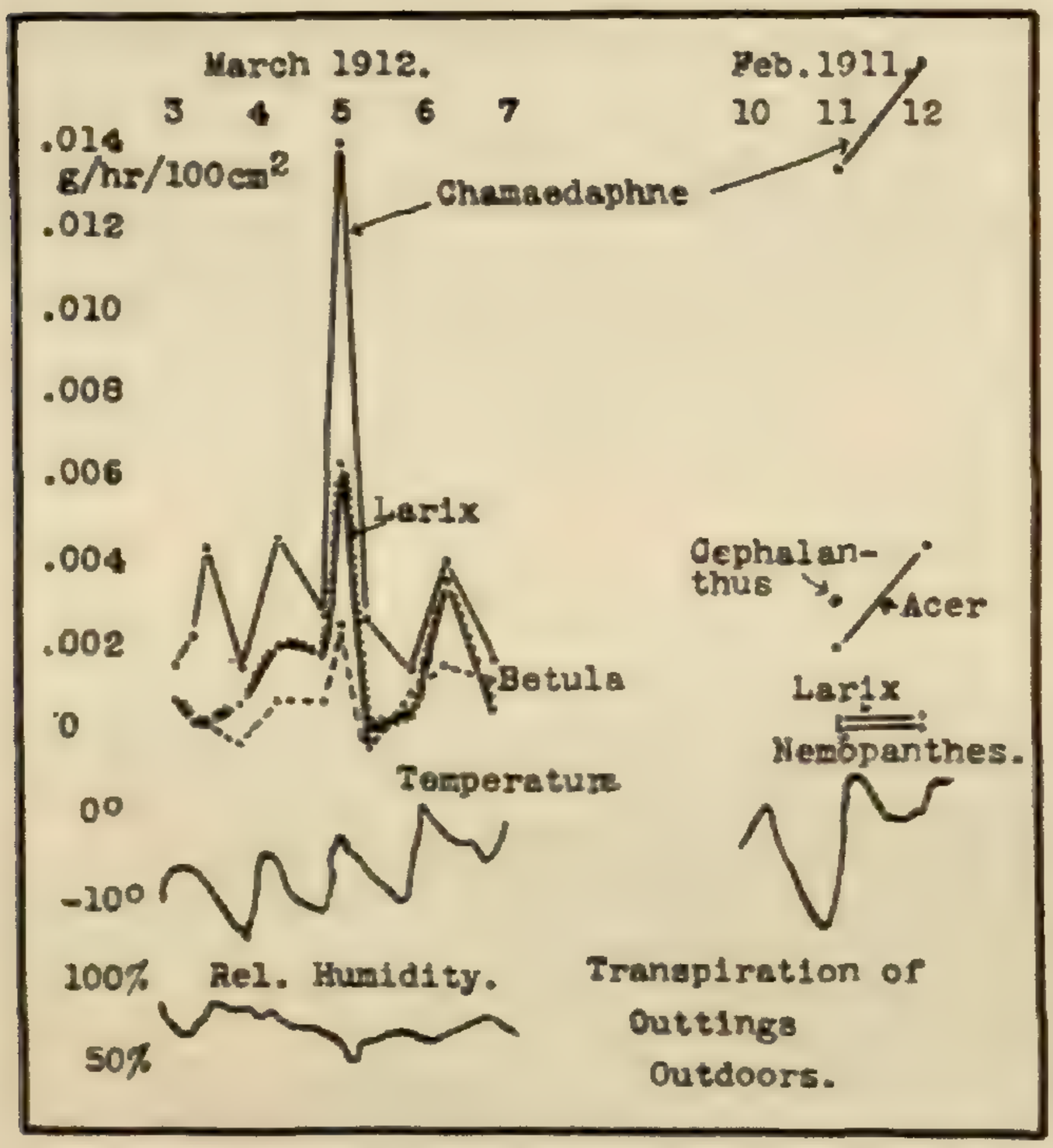


FIG. 6.—Transpiration of cuttings of *Acer rubrum*, *Betula pumila*, *Cephalanthus occidentalis*, *Chamaedaphne calyculata*, *Larix laricina*, and *Nemopanthes mucronata* outdoors in winter.

During stormy weather it is difficult to allow a real exposure to the weather and then be able to measure transpiration by weighing, but repeatedly, even when preparations were shielded from the rain or snow, measurement showed an increase in weight beyond that of a control. This was noticed particularly in *Chamaedaphne* and *Andromeda*, whose leaves were covered beneath with scales and hairs, respectively. This absorption helps replenish the saturation deficit of the leaves, and so in a measure alleviates the demands upon the root system at a time of year when absorption is at best difficult. It is not certain, however, whether this water absorbed from the air ever actually penetrates to the mesophyll.

Influence of solution temperature upon transpiration

To determine the influence of the temperature of the solution from which the cutting was taking water upon the rate of tran-

spiration, a number of experiments were made in the laboratory, the results of which are summarized in table I.

TABLE I

SUMMARIZING THE EFFECT OF LOWERING THE TEMPERATURE OF THE SOLUTION UNDER THE SAME EVAPORATING POWER OF THE AIR. THE TRANSPIRATION IS EXPRESSED IN GRAMS PER HOUR PER 100 SQ. CM., AND THE NUMBER OF MEASUREMENTS UPON WHICH THE AVERAGE IS BASED IS GIVEN IN PARENTHESES

Under room conditions (temp. 18–25° C.; rel. hum. 30–40 per cent)

	Solution at room temperature	Solution at freezing temperature
Chamaedaphne (Chamaedaphne association).....	0.062 (13)	0.054 (13)
Chamaedaphne (Carex association).....	0.080 (2)	0.068 (4)
Chamaedaphne (Larix association).....	0.042 (2)	0.042 (3)
Larix laricina (Larix association).....	0.022 (4)	0.028 (4)

Under radiator conditions (temp. 38–50° C.; rel. hum. 4–20 per cent)

	Solution at radiator temperature	Solution at freezing temperature
Chamaedaphne (Chamaedaphne association).....	0.141 (12)	0.067 (11)

In the course of these experiments cuttings were made during the cold snowy weather of February and March 1912, and subjected to different conditions in the laboratory. The laboratory conditions of temperature and relative humidity are more extreme than these plants are ever naturally subjected to in winter.

To determine the influence of a cold source of water supply, twigs were set up in water in bottles, set in a snow mixture, which kept the temperature of the solution at or near freezing. Other twigs set up in the room gave data for comparison. The general results show a lower rate of transpiration from the colder solution. The effect, however, is not so pronounced as might be expected. The control was much less perfect in leafless twigs than in leafy ones. Leafless twigs have occasionally shown a higher rate of transpiration from the colder solution. This is probably due to the stimulation which is the first effect of application of cold (BOSE 2). Such a higher rate, however, is only temporary, although it has continued for 10 hours in some of the leafless twigs experimented upon. Warming the solution was uniformly accompanied by a rise in the rate of transpiration. A lowering of the rate of

transpiration from a colder solution would be caused by the extra amount of energy required to warm the colder water up more degrees of temperature. In nature these bog plants are subjected to these extremes of temperature maintained in the laboratory, and this range is well within their physiological ability.

These experiments were repeated under the very severe conditions obtaining over a steam radiator. As the temperature of the solution became hot, transpiration proceeded at a very rapid rate, until the tissue was killed at a temperature of 42–46° C., after which the transpiration fell to a low amount without a corresponding drop in the evaporating power of the air. The graph obtained by plotting the data may be called the death curve. It is similar to those obtained by BOSE (2) in experimental work on death in plant tissues. After the marked drop, the rate of transpiration increases and then fluctuates with the evaporating power of the air, but does not exhibit the decided increase which the access of sunlight causes in living twigs.

Other twigs maintained in cold water under radiator conditions exhibited a higher rate of transpiration than those in the room. The average rate of transpiration under these conditions was very little in excess of that of twigs in the room with solution at room temperature. This clearly shows the retarding influence of cold soil water. It also shows the ability which these plants possess of withstanding a much severer aerial condition of high temperature and low relative humidity than they are ever subjected to in nature.

Rate of conduction of a 0.5 per cent aqueous solution of lithium nitrate

Table II summarizes the winter experimentation upon the rate of conduction in peat bog plants.

The results obtained from these experiments were subject to considerable variation, but in general the rate of conduction was greater from warmer solutions. Plants from habitats where the soil temperature is naturally lower seem to have a greater ability to conduct water from a colder solution. Transpiration takes place faster from a warmer solution. An increase of rate of conduction from a warmer solution is just what is to be expected to supply the greater demand for water which increased transpiration entails.

TABLE II

Under room conditions (temp. 18–24° C.; rel. hum. 27–45 per cent)

	Solution at warmer temperature (20°)	Solution at colder temperature (0°)
	cm./hr.	cm./hr.
Chamaedaphne (Chamaedaphne association)	3.1 (29)	2.5 (33)
Chamaedaphne (Carex association)	3.2 (13)	3.1 (12)
Chamaedaphne (Larix association)	3.5 (14)	4.3 (12)
Acer rubrum	0.8 (3)	0.6 (4)
Spiraea salicifolia	2.1 (2)	1.3 (2)
Betula pumila	2.4 (3)	2.0 (3)
Larix laricina	3.0 (17)	2.7 (19)

Under conditions found over steam radiator

	Solution at warmer temperature (40°)	Solution at colder temperature (0°)
Chamaedaphne calyculata	3.0 (9)	2.8 (9)
Larix laricina	5.1 (4)	.....

Experiments that were run outdoors showed a rate of conduction less than 1 cm./hr. Conduction continued to take place even after the solution was frozen.

Relationship between rates of conduction and transpiration in evergreen ericads

Tables III and IV summarize the experimental results.

TABLE III

EXPERIMENTS OF APRIL 4–6, 1912

Rate of conduction in centimeters per hour

	Chamaedaphne calyculata	Andromeda glaucophylla	Vaccinium macrocarpon
April 4	5.90 (7)	7.5 (13)	.....
April 5	4.90 (9)	.....	8.3 (12)
April 6	.....	11.1 (12)	16.3 (11)
Average	5.34	8.43	12.11
Ratio	1.00	1.58	2.27

Rate of transpiration in grams per hour per 100 sq. cm. of leaf surface

	Chamaedaphne calyculata	Andromeda glaucophylla	Vaccinium macrocarpon
April 4	0.140 (2)	0.230 (2)	.....
April 5	0.101 (2)	.....	0.301 (2)
April 6	.....	0.290 (2)	0.527 (2)
Average	0.121	0.260	0.414
Ratio	1.00	2.15	3.42

TABLE IV

CHAMAEDAPHNE CALYCULATA

	February 27	March 1	March 3	March 5
Laboratory (room temp.)...	$\frac{0.067}{2.4}=0.028$	$\frac{0.051}{1.8}=0.028$	$\frac{0.043}{2.8}=0.015$	.....
Laboratory (sol. freezing) ..	$\frac{0.052}{1.9}=0.027$	$\frac{0.052}{2.3}=0.023$	.....	$\frac{0.060}{2.5}=0.024$
Radiator (rad. temp.).....	$\frac{0.111}{2.9}=0.038$	$\frac{0.199}{2.0}=0.099$	.....	.....
Radiator (sol. freezing).....	$\frac{0.073}{2.5}=0.029$	$\frac{0.61}{2.6}=0.023$	.....	.....
Outdoors (sol. frozen).....	$\frac{0.010}{0.5}=0.020$	$\frac{0.007}{0.8}=0.009$	$\frac{0.003}{0.7}=0.004$	.....

To obtain the factor in table IV, the average of the values of all specimens under the same conditions during a day was used as a unit. The rate of transpiration was divided by the rate of conduction. The resulting factor expresses the number of grams of transpiration per unit area, which is equivalent to 1 cm. of conduction. With the solution at a lower temperature in one of two simultaneous sets, but the evaporating power of the air the same, it takes smaller amount of transpiration to account for 1 cm. of conduction.

Utilizing the high temperature of the steam radiator in the laboratory, it was found that a much greater (2–4 times) amount of water loss occurred in proportion to the amount of conduction in plants kept in solutions at the radiator temperature than near freezing. The temperature of the radiator exceeded the death point of the plants and was far above any temperature that their roots are ever subjected to in nature.

Plants maintained in solutions near the freezing point in the extreme evaporating condition of the air above the radiator, a condition which is more severe, with respect both to summer heat and to summer soil water temperatures, than any ever experienced by *Chamaedaphne* in its natural habitat, exhibited a higher transpiration and a higher rate of conduction than plants kept in the room at both room and freezing temperatures. This was in spite of the cold solution. A comparison of the transpiration-conduction factor of plants in freezing solutions in the room and over the radiator shows scarcely any difference.

With cuttings experimented upon outdoors it took even less transpiration for a given amount of conduction.

The logical conclusion substantiated by these data is that the ability of plants of *Chamaedaphne* to conduct water is at all times, when above -15° C. (the lowest temperature at which experiments were performed), greater than the necessity, and that xerophytic modification in any part of the plant is not a result of the lack of ability to conduct water.

The occurrence of temperatures of -15° is frequent in the natural range of these plants and no injuries are apparent. At a temperature lower than -25°, such as occurred during February 1912, the drying up of the exposed leaves, twigs, and flower buds of *Chamaedaphne* is first hand evidence that at such a low temperature the conduction in the upper part of the plant is not sufficient to supply the transpiration demands.

Relation of winter to the xerophily of peat bog ericads

Among peat bog plants, ericads, with but few exceptions, retain their leaves during the winter, when most other plants are leafless. This is usually considered a matter of heredity. The presence of leaves very materially increases the evaporating surface of the plant and thereby enhances the demand for water from the habitat.

That the different ericads experimented with differed among themselves in their transpiring ability with their degree of protection from excessive evaporation during the winter, and that *Chamaedaphne*, the least protected, transpires considerably more than the leafless shrubs and trees, indicates both the effectiveness of the deciduous habit as a xerophytic adaptation and the necessity of there being other xerophytic modifications in the case of the ericads. Added to this, the difficulty of absorbing water is apparent, due, if for no other reason, to the colder temperature of the bog soil in winter, and the necessity of xerophytic modification. The presence of so many of the usual xerophytic modifications in these peat bog ericads is noteworthy. The thick cuticle, dense palisade layer, more or less sunken stomates, hairs, scales, bloom, and waxy coverings, resin, and the upright position of the leaves are all indications

of this xerophily, this necessity of keeping the transpiration within the limits of absorption and conduction.

From the experimental work it was evident that, supplementing any water vapor absorbed by the leaves, the rates of absorption and conduction in the evergreen ericads are more than ample to furnish sufficient water for transpiration during warm spells in winter and for continued periods of cold temperature down to at least -15°. When, however, continued temperatures lower than -20° prevail in this region, the rate of conduction is not sufficient to prevent drying and death of the exposed parts of the plant. On this point the winter of 1911–1912 gave remarkable evidence. Consequently, a high degree of xeromorphism is demanded to compensate for a lack of a decrease of leaf surface during the winter.

EXPERIMENTATION DURING THE SUMMER

Transpiration

The water loss of plants is greatest in amount during the summer or growing season. The greater evaporating power of the air, the greater exposure of surface of most plants, the greater availability of water, and the use of water in photosynthesis all express the direct opposite of the conditions in winter and show the greater need for water in the summer. The results of a few typical experiments have been plotted in figs. 7–9.

During the first week of July 1912, the transpiration of three series of potted plants was determined at frequent intervals (fig. 10). As this week was one of extremely hot, dry weather, it will serve to indicate the limits of transpiration to which these plants may be subjected in summer. At the beginning of each series of potted plants a number of cuttings were also run to enable a comparison between cuttings and potted plants of the same species.

With the potted plants it is noteworthy that, with but a single exception, the period of maximum transpiration was reached at the time of maximum evaporating power of the air, a little before the middle of the afternoon. The graphs of evaporating power of the air and those of transpiration of potted plants are very similar. Per unit area, however, between three and five times as much

water is evaporated from a free water surface as from leaf surface on hot days.

With cuttings somewhat different results were obtained. With occasional exceptions, especially on less extreme days, the maximum daily transpiration was reached before noon, 2–4 hours before that

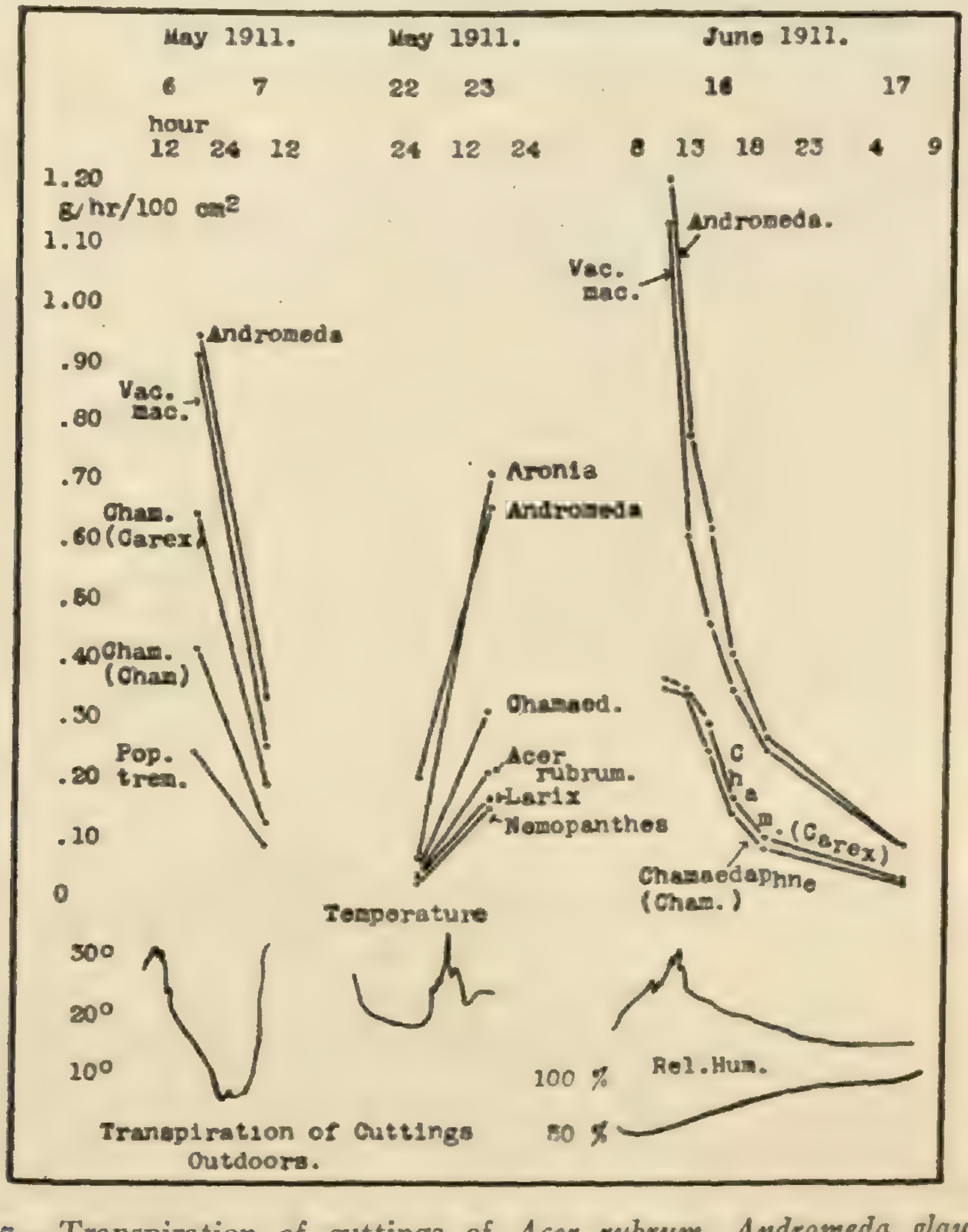


FIG. 7.—Transpiration of cuttings of *Acer rubrum*, *Andromeda glaucophylla*, *Aronia melanocarpa*, *Chamaedaphne calyculata* (from both the *Carex* and the *Chamaedaphne* associations), *Larix laricina*, *Nemopanthes mucronata*, *Populus tremuloides*, and *Vaccinium macrocarpon* outdoors.

of the maximum evaporating power of the air. Exceptions were more general in shrubs and especially in *Chamaedaphne*. In this species, whose rate of transpiration is low at all times, even in cuttings, the maximum transpiration accorded closely with the maximum evaporating power of the air.

A comparison of the results of potted plants and cuttings of the same species, although exhibiting some variation, shows that the rate of transpiration is greater in the potted plant. The difference is usually greatest at the time of maximum evaporating power of the air. Under less extreme conditions of evaporation, the

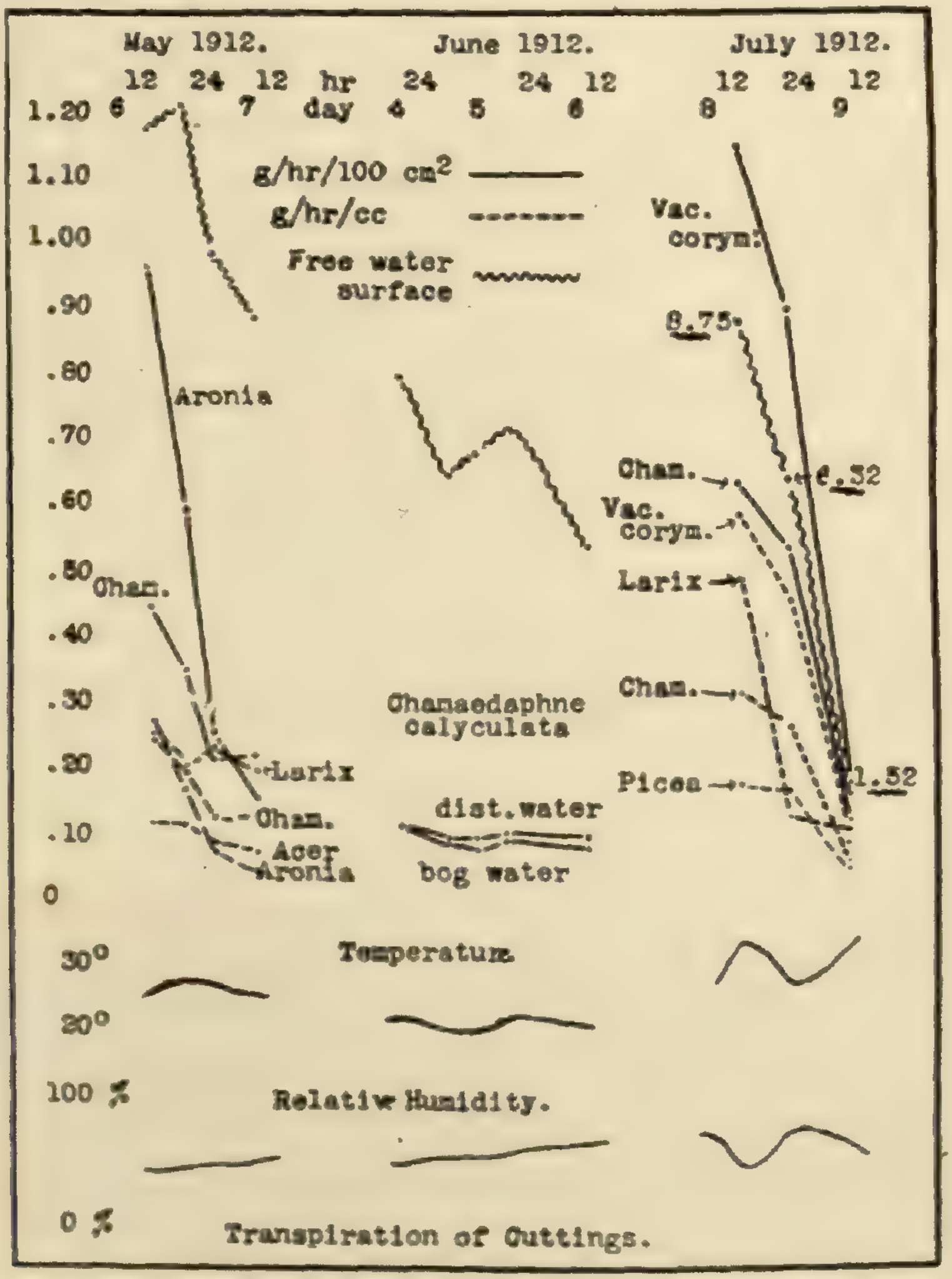


FIG. 8.—Transpiration of cuttings of *Acer rubrum*, *Aronia melanocarpa*, *Chamaedaphne calyculata*, *Larix laricina*, *Picea mariana*, and *Vaccinium corymbosum* outdoors, and of cuttings of *Chamaedaphne calyculata* from bog and distilled water in the laboratory.

graphs of transpiration of cuttings accord more closely with those of potted plants. From this it follows that one can obtain a knowledge of relative values under moderate conditions with cuttings, but experimentation under extreme conditions of evaporating power of the air with cuttings is unsatisfactory.

From an inspection of all of the summer data, it is seen that

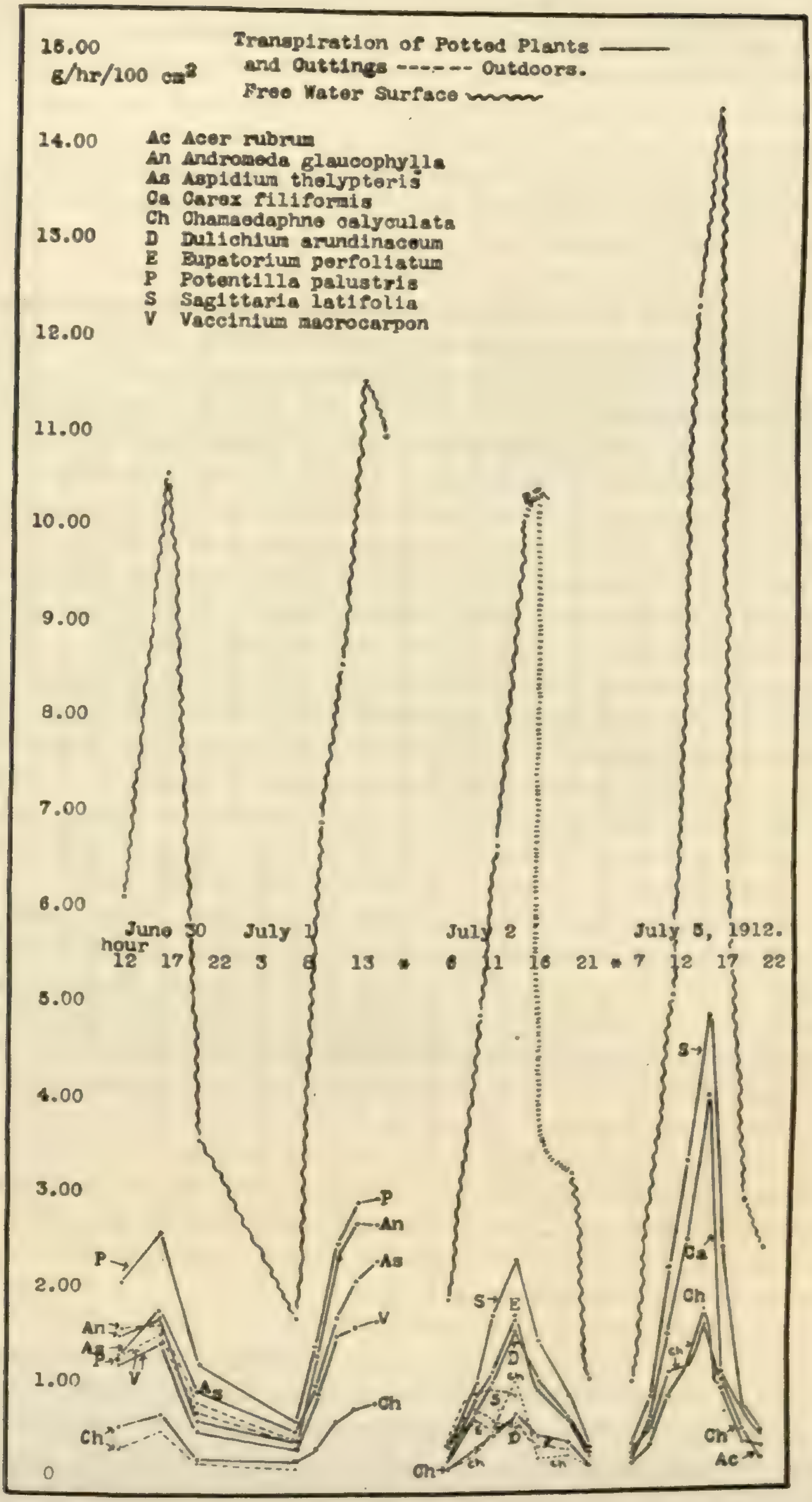


FIG. 9.—Transpiration of certain potted plants and cuttings during a severe hot wave.

among the shrubs, *Chamaedaphne* exhibits the lowest rate of transpiration. Herbaceous plants in general show a higher rate of transpiration than shrubs, while trees usually have a lower rate. Among plants that may be exposed to winter conditions, evergreen trees and shrubs transpire at a lower rate than deciduous trees and shrubs.

FIG. 10.—A view of potted plants and cuttings in the course of a transpiration experiment during a hot wave; July 2, 1912.

Rate of conduction

For use in these experiments cuttings were made in the evening after the stomates had closed, and kept under laboratory conditions over night, where the sun could strike them in the early morning, after which the experimentation was carried on as usual. Consequently, the results are not maximum rates of conduction, but relative rates of different species under similar conditions. In the summary of this work (table V), each set of

experiments is grouped together, and each figure represents at least two determinations.

TABLE V

SUMMARY OF SUMMER EXPERIMENTATION (1912) ON RATE OF CONDUCTION OF PEAT BOG PLANTS; RESULTS IN CENTIMETERS PER HOUR

	July 6	July 6	July 8	July 9
Temperature	31°	33°	25°	26°
Relative humidity	59 per cent	48 per cent	69 per cent	69 per cent
Chamaedaphne calyculata	12	20	13	22
Andromeda glaucophylla	21	.....	.....	.....
Vaccinium macrocarpon	23	.....	.....	.....
Vaccinium corymbosum	.....	.....	60	.....
Larix laricina	(31*)	.....	47	.....
Picea mariana	.....	.....	26	.....
Acer rubrum	.....	70	.....	.....
Cephalanthus occidentalis	(27*)	(140*)	.....	(170*)
Aronia melanocarpa	(51*)	.....	.....	108
Salix pedicellaris	(35*)	.....	.....	.....
Nemopanthes mucronata	(40*)	.....	.....	.....
Spiraea salicifolia	.....	48	.....	.....
Sambucus canadensis	.....	.....	.....	180
Aspidium thelypteris	(30*)	.....	.....	66
Asclepias incarnata	.....	.....	.....	210
Eupatorium perfoliatum (wilted)	.....	.....	.....	84
Sagittaria latifolia (wilted)	.....	.....	.....	49
Hypericum virginicum	.....	.....	.....	126
Potentilla palustris	.....	.....	.....	86
Typha latifolia	.....	.....	.....	114
Carex filiformis	.....	.....	.....	90
Sarracenia purpurea	.....	.....	.....	102

*Figures followed by an asterisk and inclosed in parentheses indicate that the lithium nitrate had reached the tip of the stem within the time of experimentation.

From an inspection of the data of table V it is readily seen that the evergreen ericads experimented with are all characterized by a low rate of conduction, decidedly low in comparison with that of both herbaceous plants and other shrubs. The rate of conduction was markedly lower in the evergreen bog trees than it was in the deciduous bog trees. Southern swamp shrubs showed a higher rate of conduction than northern bog ones.

Relation of summer to the xerophily of peat bog ericads

During the summer all the peat bog plants are actively carrying on the work of the growing season and have their greatest need of water. Especially at the beginning of the growing season the soil

water is cold, more so in the tree and shrub associations than in the herbaceous ones. This necessitates conservation of water and consequently xerophytic adaptation. Leaves that are produced in a time of drought (June 1912 at Mud Lake) are more xerophytic, in that they remain upright, than those produced under normal conditions (May 1912 at First Sister Lake, fig. 11). Neither observations in the extreme summer drought of 1911 in Cheboygan

FIG. 11.—*Chamaedaphne calyculata* in bloom at First Sister Lake; May 6, 1912

County, Michigan, nor experimentation under severer conditions than obtain in nature made it apparent that such a degree of xeromorphism as exhibited by *Chamaedaphne* was necessitated by the summer conditions alone.

ABSORPTION OF WATER VAPOR FROM A SATURATED ATMOSPHERE

From the increases in weight repeatedly obtained while experimenting with potted plants and cuttings of ericads during times of high relative humidity, it seemed necessary to assume the ability to absorb a measurable amount of water from the water vapor in the air, at a time when the evaporating power of the air was low. During June and July 1912, some measurements were made of the

changes in weight which took place when leafy twigs were subjected to dry air and moist air under bell jars. A twig of *Chamaedaphne* which lost water at the rate of 0.026 gm. per hour per 100 sq. cm. of leaf surface in dry air, while under a bell jar with a dish of water gained at the rate of 0.010 gm. Twigs of *Picea mariana* and *Larix laricina* gained in weight in moist air at the rate of 0.00027 and 0.000034; while those in dry air lost at the rate of 0.0061 and 0.0111 gm. per hour per cc. of volume, respectively.

These experiments demonstrate the ability of the living leaves of the three evergreen ericads and two conifers used to absorb water vapor in measurable quantities from saturated air. The amount so absorbed has increased the weight of a leafy twig by 6 per cent in 4.5 hours. When one considers the low position of bogs and the presence of heavy dew practically every night, no inconsiderable quantity of water may be accumulated in this way and furnish a part of the water for evaporation during the day and so lessen the demand upon the roots.

Dead leaves of *Chamaedaphne* absorb considerably more (3–6 times) than living ones. The scales which are present in abundance on the lower sides of the leaves no doubt take an active part in this absorption. Their structure is very similar to those of certain epiphytic orchids (*Vriesia*) which have been shown to be water-absorbing.

This function may serve to explain the excessive abundance of scales produced on the leaves developing in the excessively dry month of June 1912 at Mud Lake, in opposition to the smaller number on the leaves of the plants at First Sister Lake which developed in May before drought conditions set in.

The lessening of the drain upon the root system, when absorption is at best difficult, is no doubt greatly assisted by vapor absorption by the leaves. The prevalence of dew and frost in the bogs even when absent on the uplands indicates the greater humidity of the bog conditions. The actual presence of snow in contact with the leaves greatly aids in two ways: in checking water loss, and in constantly furnishing water vapor which may be absorbed.

The effect of absence of snow protection in time of cold, dry weather was admirably shown in the vicinity of Ann Arbor during

the winter of 1911–1912 (GATES 19). The bushes of *Chamaedaphne* were uniformly killed down to the snow level. This might have been due to the cold itself, but more likely was the result of excessive drying beyond the limits of the plants to recuperate. The difference in temperature at the snow level and 3 cm. below it was scarcely noticeable, upon the occasions when it was determined, during the moderately cold weather (−15 to 20°), yet above the snow line the plants died. At low temperatures the absolute amount of water vapor that can be in the air is very low, but below the line of snow the air is saturated. This argues for death by drying rather than by freezing, for the leaves below the snow line have more chance to keep within the drying limits of their protoplasm.

EXPERIMENTATION UPON THE CONDITION OF THE STOMATES

As has already been outlined, the data obtained upon this question are based upon the time it took a penetrating liquid (xylol) to render the leaf translucent. Different species varied in the readiness with which they became translucent, but a little experimentation soon sufficed to disclose a "normal" rate upon which to base deductions.

During the entire course of the experiments upon the several species of bog plants, the relative time of penetration indicated that the stomates were always open during the time that the full sun shone upon them. For many plants the diffuse light of early morning was sufficient to open the stomates partially, but it did not always appear that they were wide open until the full sun shone upon them. In other species, as *Nymphaea advena*, the stomates did not open until the direct sun reached the leaves. So far as was observed, clouds did not cause the closing of the stomates on hot, dry days, but on cloudy days the stomates closed earlier in the afternoon than on sunny days.

Just after the time of maximum transpiration and maximum evaporating power of the air, the stomates begin to close. The rate of transpiration drops very quickly, but stomates close slowly, and in most of the species are not completely closed until after dark. In a few species, as *Salix pedicellaris*, there was no evidence that the stomates ever closed.

Plants that were collected in the morning always had their stomates wide open at the time of collection. Before the laboratory was reached, however, the plants were frequently wilted, and investigation always showed the stomates closed. If not too severely wilted, the plants survived within an hour after cutting under water under laboratory conditions of diffuse light. When revived, the stomates opened partially in the diffuse light, but opened wide when exposed to sunlight.

Collections in the early afternoon of summer days were abandoned because of the difficulty of getting the material into the laboratory without extreme wilting. At this time of day the stomates were always wide open in the field, but closed upon wilting.

Collections made just before dark showed different results for different plants. The sun no longer shone upon the leaves, although it was by no means dark. Most of the leaves were very slowly penetrated by the xylol, which indicated that the stomates were but partially open. In some species (*Nymphaea*) the stomates were closed tight and no penetration would take place even in two minutes.

Some experimentation was performed on material kept in the laboratory with the following results. Wilted plants (*Aspidium thelypteris*, *Dulichium arundinaceum*, *Menyanthes trifoliata*, *Nymphaea advena*, *Potentilla palustris*, *Eupatorium perfoliatum*, *Acer rubrum*, *Spiraea salicifolia*, and *Vaccinium macrocarpon*) invariably had their stomates closed, as the length of time it took the xylol to penetrate the leaves testified. One single exception was found to be *Salix pedicellaris*, in which there was a scarcely perceptible increase in the time of penetration of wilted specimens as compared with plants in the field. If wilting was allowed to continue until the leaves became dry, the almost instantaneous penetration gave proof of the open condition of the stomates in the material used (*Spiraea salicifolia*, *Nemopanthes mucronata*, *Aronia melanocarpa*, *Salix pedicellaris*, *Chamaedaphne calyculata*, *Potentilla palustris*, *Ilex verticillata*, *Cephalanthus occidentalis*, *Menyanthes trifoliata*, *Carex filiformis*, and *Larix laricina*). Those of *Gaylussacia baccata* were only partially open.

Leaves of *Salix pedicellaris*, *Andromeda glaucophylla*, *Chamae-*

daphne calyculata, *Aronia melanocarpa*, *Potentilla palustris*, *Acer rubrum*, and *Cicuta maculata* were kept in the laboratory window submerged in water for a couple of days. Xylol determinations disclosed the fact that the stomates were open both day and night. Only in *Chamaedaphne* was there a perceptibly longer penetration time after dark than in the day.

This method of experimentation upon living plants in the field, which gives results so very quickly, leads to the view that stomatal movements and variations of the rate of transpiration are to a high degree independent of each other. Light is the fundamental cause for change in the first and the evaporating power of the air in the latter. The fact that the maximum evaporating power of the air occurs during the period of light does not necessarily mean that the stomates regulate transpiration. It has been shown that transpiration can change independently of stomatal movement (LLOYD **32**). BROWN and ESCOMBE (**53**) have shown that the outward diffusion of water vapor observed is not as great as the capacity of the stomates will permit. The check in transpiration during the day comes with decline of the evaporating power of the air, and the stomates begin to close *soon after*, with the diminishing light intensity. There is no doubt that closed stomates retard water loss, and that stomatal movements and transpiration changes often occur in conjunction; but that is not proof that one is the cause and the other its effect.

Conclusions

Water loss was demonstrated in every plant experimented with during the winter as well as during the summer. During the low temperatures of winter this water loss was very low, although it continued to take place even when the water was frozen around the stem. Either this water loss was a permanent drain on the water content of the stem or, as the experiments on conduction showed, there was a certain amount of renewal from the external frozen solution. Whether a plant can obtain water directly from an ice surface is not known, but it has been shown by KOSAROFF (**25**) and others that plant roots can absorb water vapor when the temperature is below freezing. The amounts, though small, are

usually sufficient to satisfy the needs of the plants. In nature, the ground, although often frozen to a slight depth, is usually protected by a snow cover during the winter (fig. 12). The spaces between the snow particles are saturated with water vapor. This produces a blanket effect, greatly reducing the temperature changes beneath the snow level and tending to keep the temperature nearer the freezing point in times of cold. Evaporation from the snow also furnishes an available form of water in winter.

FIG. 12.—A view in the Mud Lake bog when *Chamaedaphne* was buried under 80 cm. of snow; March 26, 1912.

The evergreen ericads are distinctly grouped apart from the deciduous plants. The transpiration of the former in winter was 2–15 times as great as in the latter. The evergreen habit, even with a noteworthy accumulation of recognized xerophytic structures, was not as efficient a protection as the deciduous habit. This the universal killing of the plants of *Chamaedaphne* above the snow line in the extremely severe winter of 1911–1912 demonstrated, while other evergreen plants, not so intensely xeromorphic, but entirely covered with snow, were unharmed.

During the summer the water loss was much greater than in the

winter, especially, to be sure, in plants of the deciduous habit. Transpiration bore a rather definite relation to the evaporating power of the air. This relation varied both with species and with individuals. Its numerical value was from one-third to one-twelfth that of the air. It was obvious how efficient the xeromorphic structure of the evergreen trees and shrubs is in lowering the rate of transpiration during the summer. During the winter the absolute value of transpiration of *Chamaedaphne* varied up to 0.02 gm. per hour per 100 sq. cm. of leaf surface as a maximum under outdoor conditions, and 0.07 gm. under indoor conditions, and did not exceed 0.30 gm. under conditions above a hot radiator, which were severer than ever experienced in nature. Transpiration from distilled water was 1.09 times that from bog water. During the summer the normal rate at night is less than 0.10 gm., while in the daytime it approaches 0.80 gm. Only on extremely hot days with low relative humidity did the rate exceed 1.00 gm. The maximum recorded was 1.70 gm./hr./100 sq. cm. This means that the average summer rate of transpiration in *Chamaedaphne calyculata* is 25–30 times greater than the maximum winter rate, while the maximum summer rate was over 80 times that of the maximum winter rate. For deciduous plants the difference is very much greater.

The highest rates of water loss were in the more hydrophytic plants, *Nymphaea advena*, *Sagittaria latifolia*, and *Carex filiformis*, which agrees with the experimental data obtained by OTIS (**36**) by a different method. In a potted plant of *Sagittaria* the maximum rate obtained during the investigation occurred on the afternoon of July 5, 1912 (4.82 gm./hr./100 sq. cm.).

As has been stated above and is here briefly repeated, during the winter the transpiration and rate of conduction of water are much higher in the evergreen plants (*Chamaedaphne calyculata*, *Andromeda glaucophylla*, *Vaccinium macrocarpon*, and *Picea mariana*) than in the deciduous ones. In the summer the rate of transpiration and conduction in the herbaceous plants (*Nymphaea advena*, *Sagittaria latifolia*, *Eupatorium perfoliatum*, *Asclepias incarnata*, *Dulichium arundinaceum*, *Aspidium thelypteris*, *Hypericum virginicum*, *Carex filiformis*, *Cicuta maculata*, and *Potentilla*

palustris) and in the deciduous woody plants (*Aronia melanocarpa*, *Nemopanthes mucronata*, *Salix pedicellaris*, *Cephalanthus occidentalis*, *Spiraea salicifolia*, *Gaylussacia baccata*, *Vaccinium corymbosum*, and *Larix laricina*) is much higher than in the evergreen shrubs and trees. The more xeromorphic the structure of the leaves the lower is the transpiration, and the more exposed to winter conditions the more xerophytic is the structure. This argues well for the winter conditions as being the fundamental causes necessitating xerophily in the evergreen ericads. The difficulty is in not being able to obtain water sufficiently fast or in sufficient quantities to supply the demands of transpiration. This is very much accentuated in the winter, mainly on account of low temperatures.

Although this xerophytic structure also reduces the water demands of these plants during the summer, there does not appear to be in summer any need of so thorough a xerophily, for neither the very extreme droughts and hot spells of the summers of 1911 and 1912, nor the extreme evaporating conditions of the laboratory appreciably injured the many plants of *Chamaedaphne* observed or experimented upon. On the other hand, the continued severe dry cold during the winter of 1911–1912 killed thousands of plants of *Chamaedaphne* down to the snow line, below which they were efficiently protected, whereas during the average winter of 1910–1911, with but very little snow on the ground during the coldest weather (barely below -17° C.), all of the bushes of *Chamaedaphne* remained alive clear to the top.

As these ericads are of distinctly northern phytogeographic affinities (HARSHBERGER **21, 22,** and TRANSEAU **48**), and consequently are less subject to the extremes of summer conditions which obtain near Ann Arbor, almost at the southern limit of their range, and are more subject to severe winter conditions in nature farther north, and as the evergreen habit is a hereditary character, the xerophytism is real and has been brought about and is necessitated primarily by the winter conditions which these plants endure.

Summary

1. The determination of the rate of transpiration per unit area of leaf surface by weighing is a satisfactory approach to a knowledge

of the demands of plants for water, one of the most essential features of their environment. This unit was taken as grams per hour per 100 sq. cm. of leaf surface.

2. While the use of rooted plants is most desirable, the results obtained through measuring the transpiration of cuttings, the stomates of which are closed at the time of collecting and during the period of adjustment to the apparatus, approach closely those of potted plants, except under extreme conditions of evaporating power of the air. Experiments run in duplicate are a check upon the behavior of individual specimens.

3. Cuttings of hydrophytic plants wilt in a very short time in spite of every precaution, and experiments of any duration cannot be made upon them. Cuttings of other herbaceous plants, if properly cared for, may be used for at least 24 hours, and cuttings of shrubs and trees may be used for a still longer time.

4. From the data obtained in this investigation, transpiration varies with the evaporating power of the air, that is, increases directly with increase of temperature, decrease of relative humidity, increase of air movement, and, other factors being equal, is greater in daylight than in darkness. The last factor, however, is due to the tendency toward increased internal temperature with absorption of radiant energy.

5. The transpiration of all plants experimented with was very low in winter, yet was demonstrable during the daytime of clear days, even at the lowest temperatures (−29°). Very frequently no transpiration could be detected during the night. The decided gain in weight which frequently occurred is to be attributed to the power possessed by the leaves of these evergreen ericads of absorbing water from a humid atmosphere. A gain always occurred on frosty nights and at times of high relative humidity. Both fresh and dried twigs of the evergreen ericads and of the conifers exhibited an ability to absorb water vapor from humid air. Such absorption is much greater in quantity per unit volume of leafy twig in the evergreen conifer *Picea mariana* than in the deciduous conifer *Larix laricina*, and in the scale-covered *Chamaedaphne* than in the other evergreen ericads. As such absorption not infrequently is three or four times as great as the transpiration

during a very cold winter day, it is obvious how great is the advantage of this ability to lessen the demands upon the root and conducting systems at a time when the ground is frozen solid.

6. The transpiration per unit surface of the evergreen shrubs was very decidedly greater (4–10–30 and more times) than that of the deciduous shrubs during the winter under both outdoor conditions and indoor conditions which simulated warm spells in winter.

7. Among the evergreen ericads the relative rates of transpiration varied in the inverse order of their exposure and of their xerophytic structure. *Chamaedaphne*, which is the most exposed and the most xerophytic, has the lowest rates of transpiration and conduction.

8. During an average southern Michigan winter, protection by snow is not essential to the preservation of *Chamaedaphne*. In an extremely severe winter (1911–1912) absence of snow protection results in the killing of parts not so protected.

9. During the coldest weather (−29°) it did not appear that twigs or leaves of *Chamaedaphne* were frozen, as they were either perfectly pliable to handling or cracked as dry leaves.

10. Experimentation upon the rate of conduction by the lithium nitrate method showed a relatively higher rate at first, following the shock of cutting. Variations in external factors could not be arranged for a given plant because the shortness of the stem necessitated a short time of experimentation, and in order to obtain the data the stem had to be destroyed.

11. The rate of conduction was faster from warmer solutions, but was never zero when the solution was frozen. As the transpiration varied similarly, it seems obvious that the transpiration exercises a general regulatory function on the rate of conduction, similar to that which it exercises upon water absorption. The regulation is not exact, for in incipiently dry plants the rate of absorption and conduction is relatively greater than the rate of transpiration, while in turgid plants transpiration may be greater than absorption or conduction.

12. While the rate of conduction was relatively higher in the evergreen ericads than in other shrubs during the winter, leafless

twigs of *Larix* exhibited virtually as high a rate of conduction, especially from colder solutions.

13. From the experimentation it appears that the rate of conduction is ample to the needs of these plants for temperatures above -15° (and probably above -20°).

14. The transpiration of all plants experimented with in summer was very decidedly greater than in winter. This was most marked in deciduous species and least so in evergreen species.

15. Under summer conditions, hydrophytes exhibited the highest transpiration per unit area of leaf surface. In general, herbaceous plants transpired at a higher rate than shrubs. The more hydrophytic swamp shrubs transpired at a higher rate than the typical bog shrubs. The evergreen shrubs transpired at a very distinctly lower rate than the deciduous ones. Among the bog trees the lowest rates occurred in the evergreen species. The rate of the deciduous conifer *Larix laricina* was noticeably higher than that of the deciduous broad leaf tree *Acer rubrum*, and was decidedly higher than that of the evergreen conifer *Picea mariana*.

16. The rate of conduction under summer conditions was very high in comparison to that under winter conditions. This was less noticeable in the evergreen ericads than in other plants. The summer rate in *Chamaedaphne* was but 10–20 times that of the winter rate. The maximum rate obtained under conditions of experimentation which were not extreme was 23 cm. per hour in an evergreen ericad, while rates above 100 cm. per hour were frequently found in other shrubs and in herbaceous plants. An evergreen bog tree, *Picea mariana*, exhibited a distinctly lower rate of conduction than deciduous trees whether conifer or hardwood.

17. In the case of peat bog plants in nature, light (particularly sunlight) seems to be the effective factor in causing stomatal movements. Stomatal movements, while effective regulators of transpiration when they occur, do not appear so closely to regulate transpiration of peat bog plants as the evaporating power of the air.

18. In view of the fact that exposure to the very extreme summer conditions in 1911 and 1912 did not affect the vitality of the evergreen ericads, that neither did the average winter of 1910–1911, with its scanty snow covering during the coldest weather,

while the extreme winter of 1911–1912 killed the parts of the evergreen ericads which projected above the snow; and in view of the fact that the evergreen habit is hereditary, that the position of the leaves in winter is different from that in summer, and that the transpiration is decidedly less than that of deciduous shrubs and of herbaceous plants in summer but greater in winter, the xeromorphy of these plants is real xerophyty, occasioned fundamentally by the necessity of protection when exposed to winter conditions and used advantageously by these plants during the summer.

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THE MORPHOLOGY OF ARAUCARIA BRASILIENSIS

II. THE OVULATE CONE AND FEMALE GAMETOPHYTE

L. Lancelot Burlingame

(WITH PLATES XXV–XXVII AND TWO FIGURES)

In a previous paper (1), the writer has described the source of the materials used in this investigation and the methods used in their preparation. Nothing need be added to the details presented in that paper except to mention the fact that there have been wide variations of weather conditions here in the last four years during which these collections have been made. Considerable differences have been noted in the stages reached at the same dates during these years. The 1910 collections appear to be two weeks or more farther advanced than those of the next year. It is not easy to be sure of the facts in regard to this, for cones from the same tree taken on the same day often vary astonishingly. It may be, consequently, that the variations observed are purely fortuitous and would not be sustained if the observations were sufficiently numerous, although I am inclined to think they would be. If so, it would appear that a wet winter is decidedly favorable to early development. The collections of ovulate cones were made about once a week throughout three years for most of the seasons, but were made every day or two during the months of March and April.

Each ovulate cone is borne at the end of a short branch. From three to five such branches commonly occur at a single whorl of branches (text fig. 1). The rudiments of these cone-bearing branches and that of the central leafy shoot are formed within the terminal bud of a branch. They can be found by dissecting such a bud from which the daughter buds are beginning to emerge in early April or late March. I was unable to find any recognizable trace of them earlier. Although buds exist within such terminal buds earlier, I did not succeed in finding any means of distinguishing between cones and ordinary leaf buds until just before the swellings of the ovules make their first appearance.

The buds all look alike externally before this, and even on dissection are so much alike as not to be distinguishable. A branch that has borne a cluster of cones one year does not ordinarily

FIG. 1.—The tip of a fruiting branch bearing 6 young cones about 4 months old: the photograph was made about August 1; 4 of the 6 cones are borne on branches arising from the same whorl, the other 2 from the whorl below; the whorl just out of the bud consists of leafy branches only; $\times\frac{1}{3}$.

bear a crop the next season. A cone-bearing branch is usually thicker and looks more vigorous than a leafy branch in the spring.

When the cones have emerged from the terminal bud and are

clearly recognizable as such, they are about the size of an English walnut. They are now distinguishable from a leaf bud by their shape, by their lighter color, and by the more numerous and slenderer leaflike sporophylls. Pollination occurs at this stage. In 4 months they have reached the stage shown in text fig. 2. They are then 6–7 cm. long and about 5 cm. in diameter. The

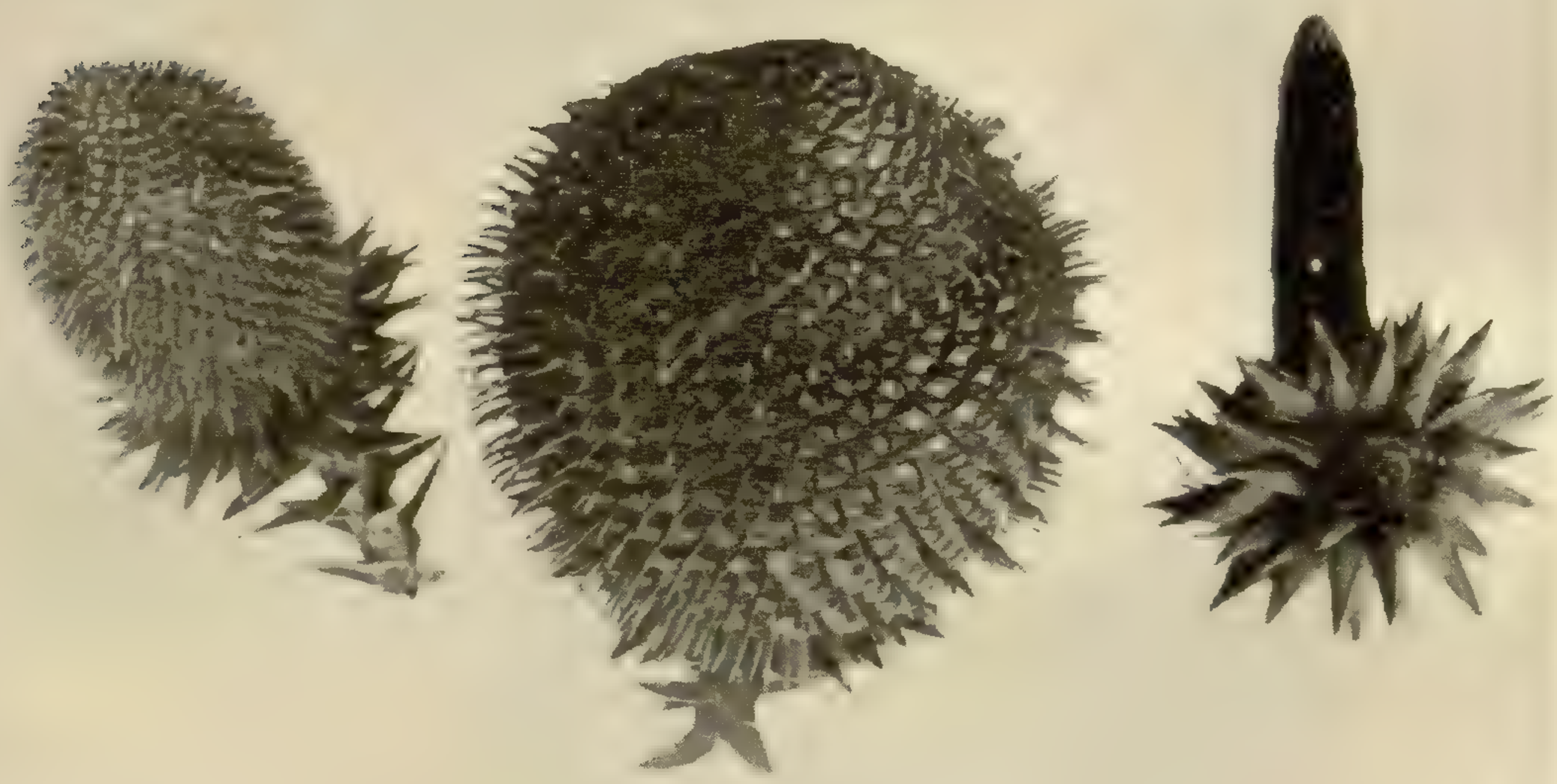

FIG. 2.—Cones of three different seasons, photographed in August: the smallest cone is 4 months old; the large cone is 16 months old; the cone axis with a few scales at base, 28 months old; the last shed its seeds in the preceding December and the largest cone would have shed seeds in the following December; $\times 1\frac{5}{4}$.

cone is rough and prickly from the turned-back tips of the sporophylls. The seeds are shed the fall or winter of the next year, when the cone has reached a length of 12–18 cm. and has shed many of its prickles. The life of an ovulate cone is thus somewhat less than two years. This is about a year less than that reported for *Agathis* (**3**), and also less than that of most conifers. There are usually 400–500 sporophylls on a cone (text fig. 2). They are arranged in steep spirals, making rather more than 1.5 complete turns.

Very soon after the cone is externally recognizable, the sporophylls show the first signs of the meristem that forms the ovule. At this time the sporophyll differs from a leaf in being differentiated into two regions. The outer part is slender and leaflike, while the basal portion is colorless, short, and stout, and tapers back slightly. The meristem is developed on the adaxial surface in a median position close to the base. It consists of many cells and grows rapidly. Figs. 1–3 show various views of it about this time. Although growth takes place throughout the sporophyll, yet four distinguishable meristems are established that determine its ultimate shape and form. The first of these is the meristem of the nucellus, already mentioned. As soon as it has made a beginning, another secondary meristem arises as a curved band across its upper surface. This curved meristematic band has its convex side directed toward the cone axis. By its growth is produced that part of the integument free from the scale. It is continuous below with the meristematic region of the scale located at its base. The fourth meristem is also a curved band, with its convexity directed toward the tip of the scale. It produces the ligule.

Not more than one sporophyll in twenty is fertile. Of those that do form ovules, many fail to mature them. Whether this tendency to sterility is equally marked in the native habitat of the tree I have not yet ascertained. It seems not unlikely that it may be due to the effects of cultivation in an alien habitat differing considerably from that of the highlands of Brazil. It has nothing to do with pollination, I am convinced, for all the cones are abundantly pollinated, both sterile and fertile sporophylls alike. The pollen tubes develop on the sterile ones, at least up to a length of 0.5 cm. or more.

The meristems very quickly produce the ovular structures. On account of this fact, and the further fact that one cannot distinguish sterile from fertile sporophylls until after sectioning them, I did not secure a complete series showing the development of the ovule. The outlines of the process, however, are clear. The basal meristem of the sporophyll elongates it, and the integument keeps pace. The result is a deep tubular cavity, deepest on the side next the sporophyll. At the base of this the nucellar meristem has been

elongating it to keep pace with the development of the integument. The result is shown in fig. 6. The line of union between the integument and the sporophyll proper is clearly indicated. In being attached along the entire side to the sporophyll, the ovule of *Araucaria* presents a sharp contrast to that of *Agathis*, which is attached only at the base (**6**).

In the nucellus three regions are at this time distinguishable. The tip consists of large clear cells, more or less isodiametric. Below this region the cells are elongated in the direction of the axis of the nucellus and arranged in fairly distinct rows. The rows become less definite toward the base. In the central portion of the base of the nucellus lies a group of cells with larger nuclei and denser contents. The megaspore arises in the midst of this group. This probably corresponds to the spongy tissue of various conifers, though it does not behave in precisely the same way during the further development of the ovule. Further reference to it will be made below.

The megaspore is picked out in a position above the bottom of the cleft between nucellus and integument. As it lies within the meristematic zone of the former, it follows that in the further growth of the ovule the female gametophyte will lie almost exclusively above the bottom of this same cleft. In this growth the nucellus does not enlarge its tip greatly. The result is that by the time the archegonia are ready for fertilization the nucellus is composed of a swollen base, containing the gametophyte, and a small extension above. The ovule at this time measures about 1 cm. in length and 3–4 mm. in diameter. The gametophyte extends through about half of it, and is about 1.5 mm. in diameter. It is somewhat oval in outline, with the archegonial end noticeably broader (fig. 4). The micropyle is shaped something like a human mouth, with its longer diameter transverse. Sometimes the upper lip is split back into a wide and deep V-shaped cleft extending back half the length of the gametophyte. Occasionally the latter breaks through the tissues of the nucellus and is openly exposed in the region of the archegonia. The further growth of the ovule gradually transforms the whole structure of ovule and sporophyll into the seed. The changes involved will be discussed

at another time. At maturity the gametophyte is 4–4.5 cm. long, 2 cm. wide, and 1.5 cm. thick. The entire seed structure is 5–6 cm. long, about 2.5 cm. wide, and 1.8 cm. thick.

The megaspore mother cell appears about the middle of May in the upper part of the group of denser cells already referred to. At first it differs only in size. As it enlarges, the adjacent cells show signs of disintegration. Fig. 5 shows a mother cell in synapsis. To the right and to the left of it may be seen two cells that are being flattened and whose nuclei are apparently beginning to degenerate already. I secured only a few preparations of the early stages and so cannot say certainly whether more than one megaspore mother cell ever begins development or not. Unfortunately I did not observe the reduction of the mother cell for the same reason. The chromosome numbers would indicate that a reduction does actually occur. Fig. 6 shows the position of the megaspore. In fig. 7 there may be seen two small cells just above the functional megaspore, that are probably the remains of the other members of the tetrad. A similar group is present in fig. 8. Very early the megaspore becomes vacuolate, with the very scanty cytoplasm forming an extremely delicate lining to the embryo sac. The nucleus is flattened, yet its thickness is several times that of the layer of cytoplasm in which it lies. It increases its volume, but does not immediately divide. Uninucleate embryo sacs are found in June, and binucleate ones (fig. 8) in July. By the latter part of the month as many as 64 nuclei may be present. Fig. 8 is that of a sac with two nuclei; in fig. 9 there are 8; and in fig. 10 a few less than 64. From this it would appear that the early divisions are simultaneous. As a matter of fact, I do not know that they are, for I have never observed a single mitosis in any of the more than 500 preparations of the free nuclear stages available for study. These preparations represent more than 50 collections of separate cones and two or three times as many separate ovules. It seems exceedingly strange that they should be so persistently missed. The same difficulty was encountered in a study made of the mitoses of the pollen mother cells (1). In that study I made the suggestion that those mitoses may occur at night. It has not yet been practicable to test out this hypothesis, and I mention it here merely

because I can think of nothing more likely. SAXTON (5) has since met the same difficulty and has hazarded the same guess. It may be that the mitoses are both simultaneous and passed through with extreme rapidity, and that it is merely chance that they have been missed. No indications of amitosis have been observed. Above the 64-nucleate stage the numbers are not regular, being usually somewhat less than the exact power of 2. Enlargement of the sac and multiplication of the nuclei continue up to the latter part of January. At this time there are more than 2000 free nuclei present. The cytoplasm still remains extremely scanty. Figs. 9–16 show the progress of development at intervals of about a month.

In January a change in the method of development occurs. Without any considerable increase of nuclei, the cytoplasm increases rapidly. As soon as it has become somewhat thicker, vacuoles make their appearance. The result (fig. 17) is that the central cavity is surrounded by a rapidly thickening sac of vacuolated protoplasm, with the nuclei largely confined to the inner border (fig. 18), In many cases the walls between the vacuoles break through. leaving the inner plasma membrane connected to the outer one merely by tenuous strands. The nuclei usually lie at the points where these strands join the inner plasma membrane. A few are found at the outer membrane, especially at the micropylar end of the sac. The protoplasm is thicker at this end also. This process goes forward very rapidly. The inner border advances on the central vacuole and the nuclei multiply somewhat. They now pass out along the cytoplasmic strands (figs. 19–21). By the time the inner border has closed up on the vacuole completely (fig. 20), most of the nuclei have migrated outward.

With the continued increase of the cytoplasm, most of it remains in the peripheral regions, especially near the micropylar end. It collects along the strands and plates until distinct uninucleate vacuolated sacs are formed. The nuclei are now generally suspended by still more delicate strands in the central portion of the sac. Fig. 22 shows how these sacs behave under the action of the killing reagents. Each sac appears to have its own wall of inclosing protoplasm capable of being separated from that of its neighbor. Mitoses now occur plentifully in preparations of the peripheral

portion of the prothallus. In this way there is formed a sort of colony of free cells closely packed together but yet capable of easy separation. This condition gradually passes into the vacuolated condition of the inner part of the gametophyte, in which region walls are not formed for some time. In fact, it never becomes so solid as the outer parts.

In about a month delicate walls have made their appearance between the peripheral cells (figs. 31, 32), though no definite cells have yet been organized centrally (fig. 25). The exact method of wall-formation was not made out. The process is remarkably suggestive of the way in which walls are formed in cleavage furrows in certain lower plants. One such cell is formed for each nucleus. After the formation of these first walls, the succeeding ones in the outer part are laid down on cell plates formed on the spindles in the ordinary fashion. The outer cells are generally uninucleate, while the central cells, after they have become walled off, become multinucleate by the time the archegonia are mature. They also later contain considerable quantities of starch.

In the further growth of the gametophyte, walls are formed on the spindles in the outer portion after each mitosis; in the central region wall-formation does not occur at this time. The transition from one region to the other is very gradual, and the walls thin out so gradually that it is almost or quite impossible to tell where there are actual walls. In fig. 31 are shown mitoses of both sorts. The one near the archegonium initial will have a wall formed on the spindle, while the one near the opposite border will not. Cell plates are formed in connection with the mitoses even before any walls are being formed on them (fig. 24). In the central region the nuclei are sometimes situated at the intersection of the larger strands and plates of cytoplasm and sometimes are suspended by much finer strands in the central region of the vacuolated spaces. Sooner or later these spaces, like the peripheral ones, form walls in the inclosing plates of cytoplasm and become cells. At the time of their inclosure by walls, they are commonly uninucleate. Later they frequently contain as many as 4–6 nuclei. Up to the time of the maturity of the archegonia the cytoplasm in all the cells of the gametophyte remains very scanty.

The outline of the growing prothallus may remain smooth and even (fig. 36) or become very irregular (fig. 35). The irregularity appears to be entirely due to two facts. The gametophyte, as already mentioned, is very delicate and plastic, and, in consequence, able to adapt its form to the cavity in which it grows. The second fact is that the cavity in which it is forming does not always enlarge regularly. Whether the irregularity is due in whole or in part to the action of the gametophyte is somewhat doubtful, as will be pointed out in the succeeding paragraphs. The development of the gametophyte beyond the fertilization stage will be further described in connection with the maturity of the ovule and the organization of the seed.

The cells surrounding the megaspore have already been mentioned as being larger, having larger nuclei and denser contents than other nucellar cells. They perhaps correspond to the so-called spongy tissue. The character of these cells is shown in figs. 6–8. As the megaspore enlarges, the innermost layers of these cells die and lose their contents. The walls also seem to disappear, though much more slowly. They are at first stretched out and closely compressed into a thick and compact band just outside the megaspore membrane. Inasmuch as this band of crushed cells does not appear to increase in thickness beyond a certain point, it seems reasonable to suppose that it is dissolved and perhaps used by the growing gametophyte. Immediately outside of this region of dead and empty cells there is a more or less distinct band of cells (figs. 10, 12, 13, 14) which stain more densely. This band of cells develops outward in advance of the gametophyte, keeping much the same relation to it as at first. The individual cells have more cell contents than the cells outside of the band. Their nuclei take basic stains strongly, as sometimes does the cytoplasm also. In short, they appear to be undergoing degeneration. The appearances described are such as have been observed in many other plants. The phenomena have been commonly ascribed to the effects of the gametophyte. It has been supposed that it secreted some sort of enzyme or other substance that diffused outward, killed and digested the cells, and prepared them for food for itself. It looks like a reasonable inference.

In the preceding paragraph I have described the appearance of the nucellar tissues around the growing gametophyte. I wish now to describe some of the anomalous conditions found that have led me to suspect the validity of the current accounts of the effects of the gametophyte on the nucellus. In fig. 26 is shown an apparently enlarging hole in the nucellus surrounded by the two usual bands of differentiated cells. Such ovules are fairly common. In some cases the megaspore membrane appears to be present. One might infer in such cases that the hole is the work of a gametophyte that for some reason or other has died. In most cases the megaspore membrane cannot be demonstrated; but since it is not well developed in any case, this would not appear to be an insuperable difficulty.

Fig. 27 shows a less common condition. There is no gametophyte here, nor is there any place for one; yet there is a remarkable correspondence in nucellar structure. In the center there is an enlarging mass of cells whose nuclei and cytoplasm are undergoing degeneration. The central cells are almost completely crushed; they have very little or no living contents. Outwardly the cells grade off through less and less crushed cells to a band of normal shape and size, but with densely staining contents, just as in the normal ovules. If there is no gametophyte and even no place for one, then the effects cannot be due to the presence of one. It seems that these facts admit of but one of two possible explanations: either the cells of the spongy tissue, which are possibly potential megaspore mother cells, are capable of producing the observed effects, or it is a quality of the nucellar cells themselves to behave in this fashion, regardless of the presence of a gametophyte or its antecedent archesporial tissue. A noticeable peculiarity of these cells in all cases is the thickening of their walls accompanying the death and disappearance of the protoplasm.

The development of such sterile ovules has nothing to do with pollination, apparently, for they occur regardless of whether the pollen has or has not sent tubes into the nucellus. They are relatively common and develop to advanced stages. Cones on unpollinated trees on the grounds of Stanford University develop to nearly normal size, though the gametophytes do not.

The megaspore membrane is usually thin and poorly organized.

It is variable in different ovules. In *Agathis* it is reported (3) that the megaspore membrane is thickest over the apex of the gametophyte and gradually thins out toward the archegonia in such a way as to allow the fertilization of the lower archegonia first and to protect from the pollen tubes the later maturing upper archegonia. There does not appear to be any such difference in *Araucaria*, though it is usually the case that the megaspore membrane disappears along with the band of dead cells in the region of the archegonia (fig. 4).

The time at which the pollen tubes reach the nucellus is subject to wide variations. They may do so as early as July or be deferred till late in the fall. The time at which they do so does not appear to exert any influence on the development of gametophyte or ovule, within the limits mentioned. So many tubes commonly reach and penetrate the nucellus that it is almost entirely destroyed. They usually enter through the tip, composed of large clear cells with little protoplasm, but may occasionally pass between the nucellus and integument for a very short distance before entering the former. In the cases of the large slitlike micropyles, through which the nucellus is exposed for a large part of its upper surface, the tubes ordinarily, at any rate, enter only through the tip. There do not appear to be any special peculiarities in the way the tubes penetrate the nucellus. They go fairly straight to the region of the archegonia. Occasionally one branches; a few strike the cap of dead cells over the apex of the gametophyte and then commonly turn aside. They are surrounded by a layer of dead cells somewhat like that around the female gametophyte, though it is less extensive and less regular. No indications of the breaking down of nucellar cells to make way for a pollen tube were observed unless the pollen tube was itself present to account for the effects. The nucellar cells below the tip commonly contain much starch, which largely disappears with the development of the tubes.

The uniformity with which the tubes enter the tip of the nucellus, even when a shorter and apparently more available path is present, suggests that they are attracted by a chemotactically active secretion from the glandular tip. I did not succeed in demonstrating such a secretion. There are often considerable quantities

of a slightly sticky liquid between the sporophylls, but I failed to find any evidence that it comes from the nucellar tip.

At about the time the walls are formed in the peripheral parts of the gametophyte, in the latter part of February usually, the archegonium initials become recognizable. Owing to their scanty contents they are recognizable only after they are somewhat enlarged. They vary in number from about 6 to 15 or more. They are situated in a ring around the crown of the prothallus. They do not all mature at the same time, though there does not appear to be any regular order. Commonly 5–8 mature and 3 or more of these are frequently fertilized. Within the circle the individual archegonia may stand alone (fig. 4) or they may be grouped in complexes (figs. 30, 44). Each archegonium is commonly surrounded by an individual jacket, though in some of the complexes there may be no cells at all between some of them (fig. 44).

The initial is commonly a wide U-shaped (fig. 31) or V-shaped (fig. 32) cell. It has a large nucleus and little cytoplasm. Sometimes a basal cell is cut off from this cell (fig. 33) before it becomes the actual initial. The nucleus of the initial divides and a periclinal wall separates a thin flat primary neck cell from the inner or central cell (figs. 32, 34, 37). The central cell enlarges much more rapidly than the neck cell (figs. 38–40). The latter soon divides by an anticlinal wall. This division is more frequently in the direction of its greater diameter. Each of the halves then divides into about 6 wedge-shaped cells. The nuclei of these wedge-shaped cells are invariably at the large end of the wedge. The points of the cells meet or nearly meet at the center of the neck. At this point the cells have commonly thinner walls, less cytoplasm, and a tendency to separate and leave a free passage to the egg (fig. 46). Viewed from above, the group of neck cells has either a rounded (fig. 46) or elliptic (fig. 47) outline; from the side they usually appear as a dome or cap (fig. 45).

Many variations in the form and outline of the neck occur. The size of the group as well as the number of cells in it is subject to considerable variation. The commonest arrangement is 12 cells arranged in a single tier (figs. 45, 46). Figs. 48–50 show three serial sections through a neck in which there is one extremely large cell.

The figures also show that the cells are not all in the same plane, but are placed more or less obliquely above one another. This is not unlikely due to crowding by the large cell. Many such asymmetrical necks occur in my preparations. Occasionally one cell is so large as almost to pass for a central cell. These large neck cells possess large nuclei, as may be seen from fig. 50. Occasionally there is more than a single tier of cells in the neck; fig. 51 shows a neck of 3 tiers of cells.

All the archegonial initials which I could certainly identify and all young archegonia occur in the surface layer of cells. In one preparation there were 4 cells in a row. The outer one resembled a primary neck cell. The lowest one was large and had the general appearance of a central cell. The inner of the other two was about one-third as large as the lowest and slightly larger than the second one. An imbedded archegonium might have resulted, possibly, from such an initial as this. Though the mature archegonia are very frequently displaced and overgrown by the neighboring cells, it is ordinarily easy to find the free open passages from them to the surface. I did not find any case in which it was not very probable that such a passage exists. I am fairly convinced, therefore, that there are no deep-seated archegonia in this species of *Araucaria*. I believe that all such appearances are due to displacement and overgrowth. EAMES has recently (3) expressed a somewhat similar opinion in regard to *Agathis*. I have examined a number of preparations of *A. imbricata* and have seen no deep-seated archegonia among them. The displacement and overgrowth of the archegonia is rendered very easy owing to the very delicate walls in the prothallus and to its frequently irregular outline. The more solid archegonium would thus be easily pushed into a position where the turgescent cells of the gametophyte would find an equilibrium of mutual pressures. The same fact would almost invariably lead to the crowding down into the archegonial cavity of the adjacent cells if they encountered any resistance at all in the expansion of the cavity in which the gametophyte grows.

The jacket usually consists of a single layer (figs. 42, 43) of more dense cells, which are usually uninucleate and with comparatively thin walls. The wall next the egg is somewhat thicker than the

others and is usually marked by a large thin spot, as is common among conifers. Occasional cells are binucleate, and sometimes the jacket is doubled in places (figs. 49, 50). In contrast to *Agathis* (3), the jacket is firmly united to the neck cells. In consequence of this the contents of the pollen tube pass through between the neck cells, in a manner to be described in another place.

The growth and development of the central cell resembles that of most other conifers in its general outlines. It enlarges rapidly but remains poor in cytoplasm (figs. 37–40). At first there is a single large vacuole, later there are many small ones in the more abundant cytoplasm (figs. 41, 42). At maturity there are usually no vacuoles and the cytoplasm is very dense (fig. 43). The nucleus enlarges with the development of the central cell. At first it is placed in the upper central part (figs. 39–41). It later migrates to one side (fig. 42) and nearer the neck. From here it passes to a position just below the neck (fig. 45) as if about to divide into egg nucleus and ventral canal nucleus. I was unable to find any evidence that it does divide. No mitotic figures were seen in the developing archegonium, nor were any nuclei, other than the one, ever seen before fertilization, except in one ovule. In this case a number of small nuclei were present in the upper part of what appeared to be a mature archegonium as yet unattacked by a pollen tube. It would be rash, perhaps, to assert that such a ventral canal nucleus is never cut off, even though a persistent hunt for it has failed to reveal it.

Discussion

I shall not attempt at this time to discuss broadly the relationships of *Araucaria* to other members of the Coniferales, but merely to point out wherein it resembles some of them and in what ways it differs from all of them in certain features of its ovule and female gametophyte.

One of the first points in which this species of *Araucaria* (also *A. imbricata*) differs from *Agathis* is in the length of time taken to mature seeds from the first appearance of the seed cone. From the time it can be first recognized until the seeds fall is approximately 21 months. EAMES (3) reports *Agathis* as forming the

rudiments of its seed cones nearly a year in advance of pollination, while here there intervenes scarcely any time at all. In fact, in California the greater part of the pollen is likely to be shed before there are any ovulate cones to pollinate. Observations in the native habitat might show a different state of affairs and one more nearly paralleling that of *Agathis*. Other conifers of course are known in which the total time is even shorter than 21 months, but I am not aware of any one in which the time is distributed in the same manner.

The very considerable number of free nuclei before cell formation is another character that, taken with the very large gametophyte, reminds one of more primitive gymnosperms. Gametophytes of this size are known only among the Cycadales, Ginkgoales, and some taxads (as *Torreya*). A curious feature of its development is the strange absence of mitoses in the free nuclear preparations.

The manner in which the free nuclear stage passes into a gametophyte with walled cells is apparently different from that reported for any other plant. While there is a certain resemblance to the centripetal growth with "alveoli" reported for *Sequoia* (4) and others, yet the exact method is really quite different. The most essential feature of this difference lies in the delay of walls. If walls formed between the nuclei before the beginning of the centripetal movement of the inner border of the cytoplasm and were extended *pari passu* with it, and the nuclei divided to keep pace, there would be no very essential difference. While far too little is known about the exact methods of centripetal growth and wall-formation in any considerable number of genera to make any conclusions based on this feature more than tentative, it is clear that *Araucaria* need not be excluded from relationship either with podocarps or with abietineous conifers on this account.

While there is no proof that the gametophyte invades the nucellus in *Araucaria*, neither is it proved that it does so in other genera where similar appearances are commonly observed. The homology of the spongy tissue and its functions is none too clear anywhere. The peculiar glandular nucellar tip is found elsewhere only among conifers of podocarpineous affinities. THOMPSON has made the suggestion that the method of pollination found in the

araucarians is "proto-angiospermic." It seems to me, on the contrary, that we have been altogether too ready to accept the type of ovule which has a specialized pollen chamber securely hidden away at the base of the scale, and which can be reached by pollen only by means of special devices, as a primitive type. It is scarcely credible that the first step in the evolution of the ovule and seed should have been so complex. If, however, the gymnosperms possessing this complex type evolved a seed before a cone, as in fact there is good reason to think they did, then this might have been at least one of the earlier successful types. If, on the contrary, the cone was evolved before the seed, or simultaneously with it, as it may very well have been in an apparently simple cone, what would be more natural than that the pollen should lodge in any convenient place among the scales (sporophylls perhaps) of the cone? Such an ovule would have a much better chance of survival in such a cone than if exposed on the lobe of a fernlike leaf such as those possessed by the known Cycadofilicales. I am aware that I am thus attempting to introduce an apparently ancestorless conifer, but fail to see that a search for fitting ancestors is likely to be more difficult than deriving it from unsuitable ones.

Imbedded archegonia have been reported (6) for *Araucaria*, and a comparison made with *Sequoia* in which somewhat similar conditions are said to be present (4). My own observations do not bear out the presence of such imbedded archegonia in *Araucaria*. They are superficial in origin and become overgrown by the neighboring cells. SINNOTT has recently reported (7) practically identical conditions in the podocarps. The necks are not specially noteworthy, though they show a rather closer resemblance to those reported for *Podocarpus* (7) than to those of most other conifers.

The failure to find a ventral canal nucleus is somewhat surprising in so large a gametophyte, not having advanced beyond the evolutionary stage in other respects that has been attained by conifers generally. It seems more probable that it will yet be found. Ventral nuclei have not been found as yet in one species of *Torreya* (2).

The gametophyte, therefore, appears to be neither highly specialized nor exceptionally primitive in its structure. Its large

size and numerous and large archegonia are offset by the late development of walls and their persistent delicacy, by the apparent lack of a ventral canal cell, and by the rather specialized necks of the archegonia. Probably it presents more resemblances to the gametophytes of the Taxaceae and to those of the Taxodineae than to other conifers.

Summary

1. The ovule possesses a very free nucellus with a glandular tip, a single integument adherent to the scale for almost its entire length, a ligule, a large micropyle, and spongy tissue surrounding the gametophyte.

2. There is probably a single functional megaspore, which develops into an embryo sac with about 2000 free nuclei before cell-formation.

3. Cell-formation follows on a peculiar centripetal growth of the cytoplasm and precedes wall-formation.

4. The first walls are formed on the surface of the free cells.

5. Secondary walls are formed on the spindles of the mitoses occurring in the primary cells of the peripheral regions of the gametophyte.

6. The outer cells are uninucleate, the inner ones are multinucleate.

7. The archegonia have single-tiered necks, usually, consisting of about 12 wedge-shaped cells.

8. The necks are on the surface of the prothallus but are often overgrown.

9. The archegonia may be single or occur in complexes and have a single-layered jacket.

10. A ventral canal nucleus may be absent.

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EXPLANATION OF PLATES XXV–XXVII

FIG. 1.—Longitudinal section of a young cone in which the rudiments of the ovule are just becoming visible; ×12.

FIG. 2.—Longitudinal section of a slightly older sporophyll, showing the beginnings of the nucellus, integument, and ligule; ×15.

FIG. 3.—Longitudinal section through the meristem of the nucellus; ×62.

FIG. 4.—Longitudinal section of an ovule just before fertilization, showing the position and relative size of the gametophyte and a protruding nucellus; ×10.

FIG. 5.—Megaspore mother cell in synapsis; ×900.

FIG. 6.—Longitudinal section through an ovule in the latter part of June, showing the enlarging megaspore; ×20.

FIG. 7.—A megaspore just before division, surrounded by spongy tissue; ×62.

FIG. 8.—A binucleate embryo sac in late June; ×62.

FIG. 9.—An 8-nucleate sac in July; ×62.

FIG. 10.—A 64-nucleate stage in late July; ×62.

FIG. 11.—A 512-nucleate stage in late August; ×20.

FIG. 12.—A small portion of the parietal protoplasm and nuclei in late October; ×125.

FIG. 13.—A whole embryo sac in October; ×20.

FIG. 14.—An embryo sac in November; ×20.

FIG. 15.—An embryo sac in December; ×20.

FIG. 16.—An embryo sac in January; ×20.

FIG. 17.—The beginning of centripetal growth of the cytoplasm; ×20.

FIG. 18.—The micropylar end of the gametophyte shown in fig. 17; ×125.

FIG. 19.—Centripetal growth half complete; note that some of the nuclei are now migrating outward and that there are no indications of walls; ×20.

FIG. 20.—A detail of the same gametophyte as shown in preceding figure; ×62.

FIG. 21.—The completion of centripetal growth of the cytoplasm; ×62.

FIG. 22.—Detail from micropylar end of sac of age shown in preceding figure; ×125.

FIG. 23.—A slightly older gametophyte; ×20.

FIG. 24.—A detail from preceding figure showing lack of walls and presence of free cells; ×250.

FIG. 25.—A central part of the gametophyte in early March, showing the lack of walls; from the same slide as fig. 31; ×125.

FIG. 26.—Section through an ovule in which the nucellus is growing and the cavity enlarging, but in which there is no gametophyte; ×20.

FIG. 27.—Section through an ovule without any gametophyte, but with its place occupied by an enlarging mass of cells probably derived from the spongy tissue; ×20.

FIG. 28.—Early stage of the erosion of nucellus by the pollen tubes, July; note the abundant starch; ×62.

FIG. 29.—A nucellus in December when most of the starch has disappeared and all the upper part of the nucellus has been destroyed by the tubes; ×62.

FIG. 30.—Section through an archegonial complex; note the remains of the apical cap of dead cells and the superficial neck; ×85.

FIG. 31.—Micropylar end of thallus about March 1, with archegonial initial; ×125.

FIG. 32.—Lateral portion of another thallus with young archegonium near top; ×125.

FIG. 33.—An archegonium initial just before division; ×250.

FIG. 34.—Young archegonium just after cutting off primary neck cell; ×250.

FIG. 35.—Apical end of an irregular thallus with young archegonia; ×62.

FIG. 36.—Similar thallus with smooth outline and young archegonia; ×62.

FIG. 37.—Young archegonium; neck cell undivided; ×250.

FIG. 38.—Similar archegonium with neck divided; ×250.

Figs. 39, 40.—Enlarging archegonia; ×250.

FIG. 41.—Archegonium about one-half mature, showing neck, vacuolate cytoplasm, jacket, and position of nucleus; ×125.

FIG. 42.—Nearly mature archegonium with nucleus to one side and just below neck; ×125.

FIG. 43.—Mature archegonium; ×125.

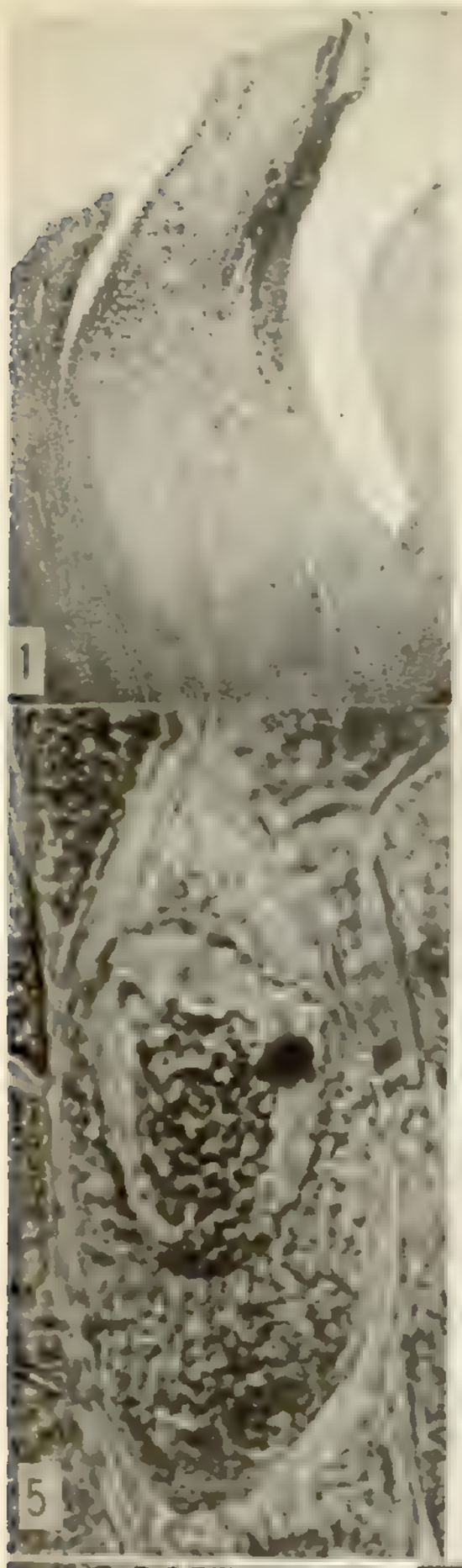
FIG. 44.—Cross-section through a complex where there are no jacket cells between two of the archegonia; ×125.

FIG. 45.—Section showing dome-shaped neck and position of nucleus below it; ×250.

FIGS. 46, 47.—Cross-sections through usual type of neck; ×250.

FIGS. 48–50.—Serial sections through neck with one very large cell; ×125.

FIG. 51.—Longitudinal section of 3-tiered neck; ×250.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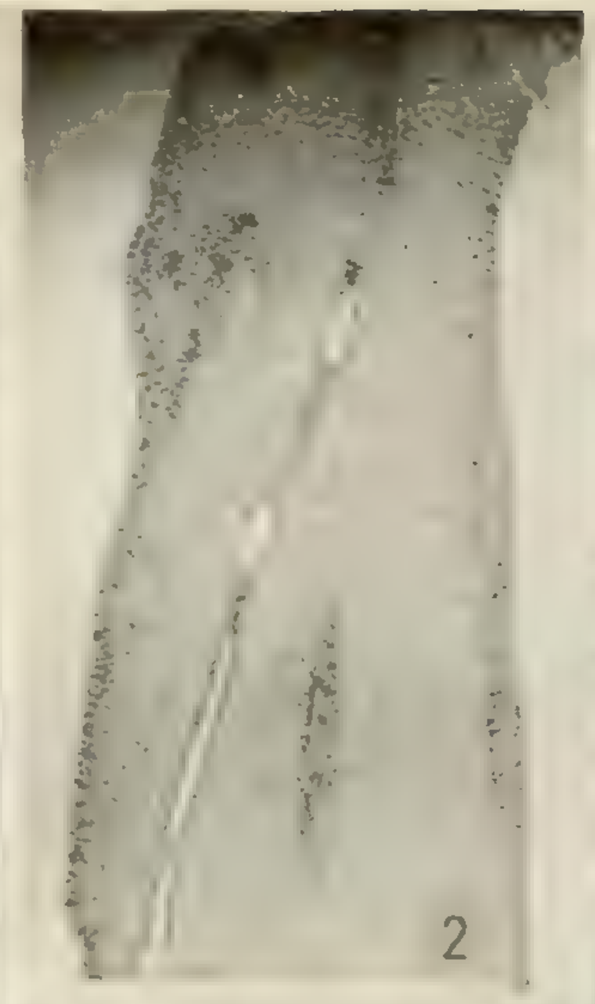
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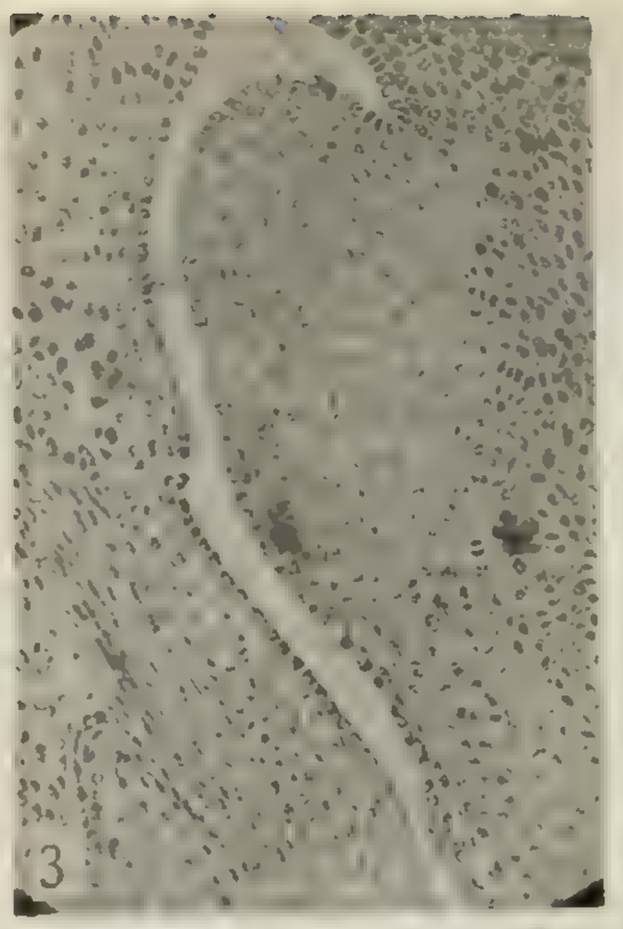
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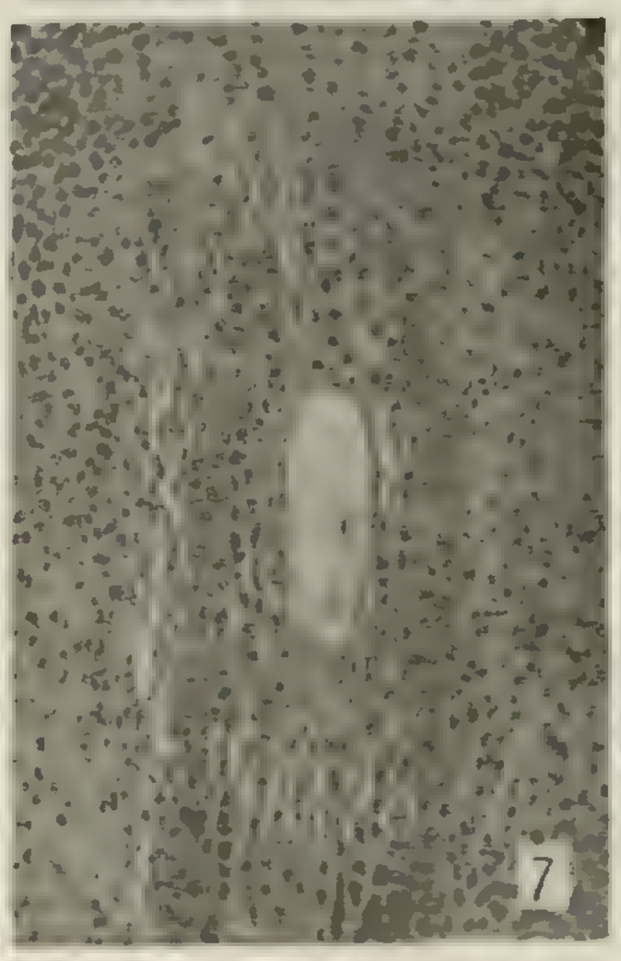
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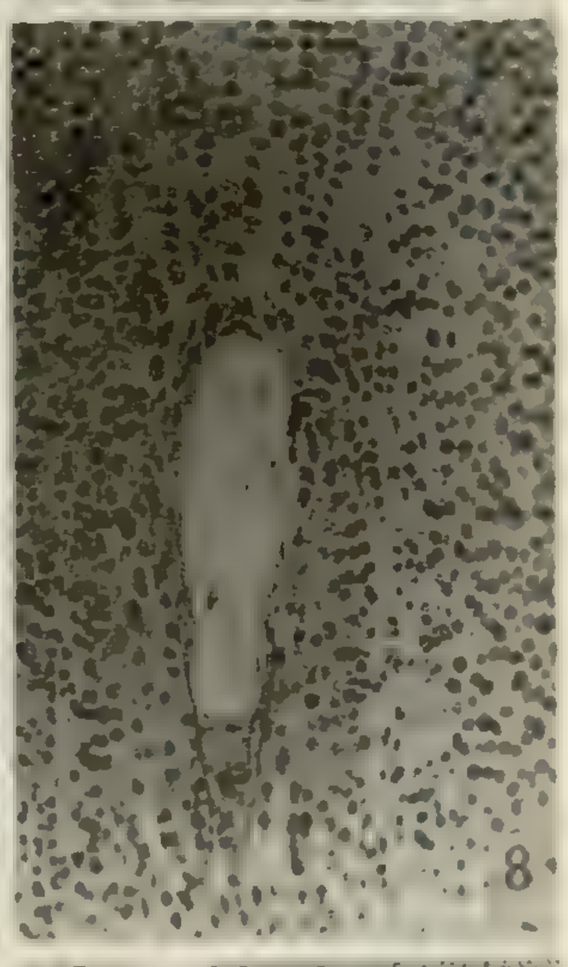
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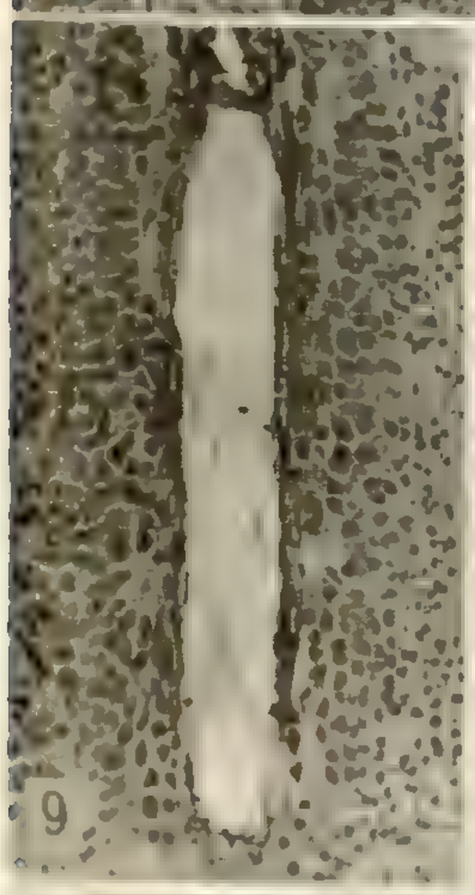
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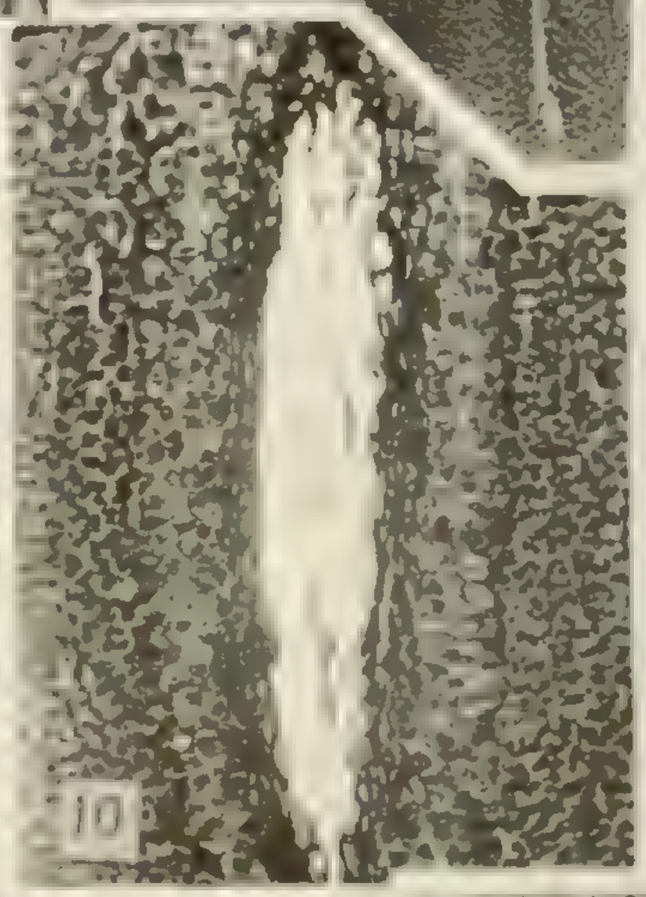
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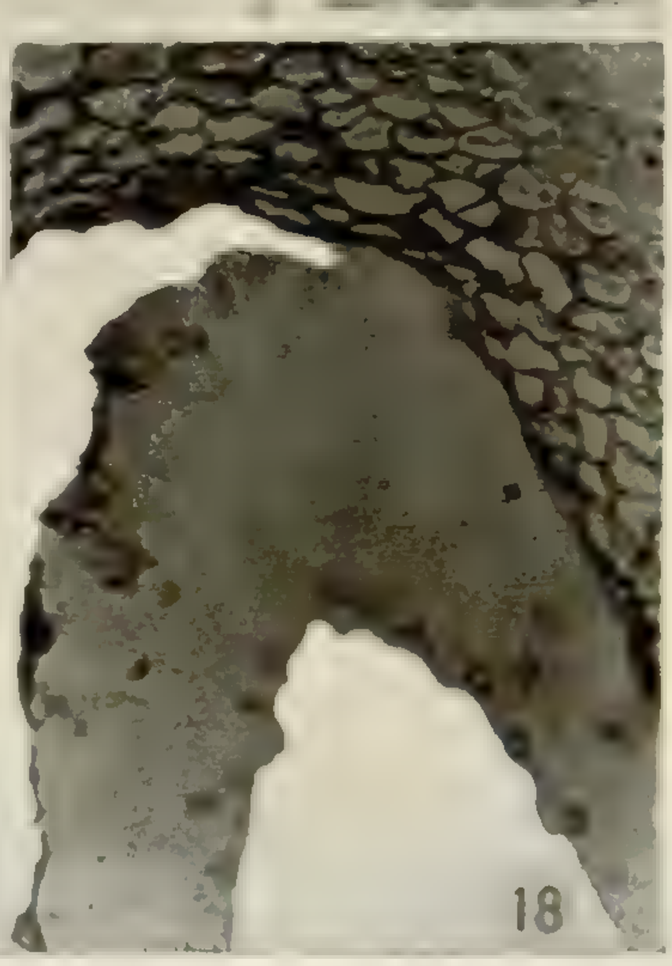
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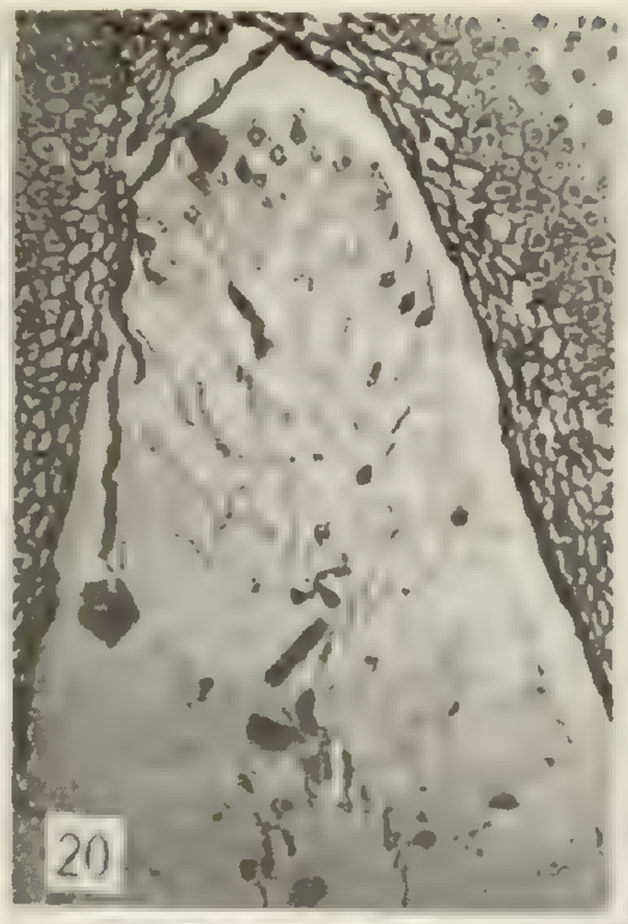
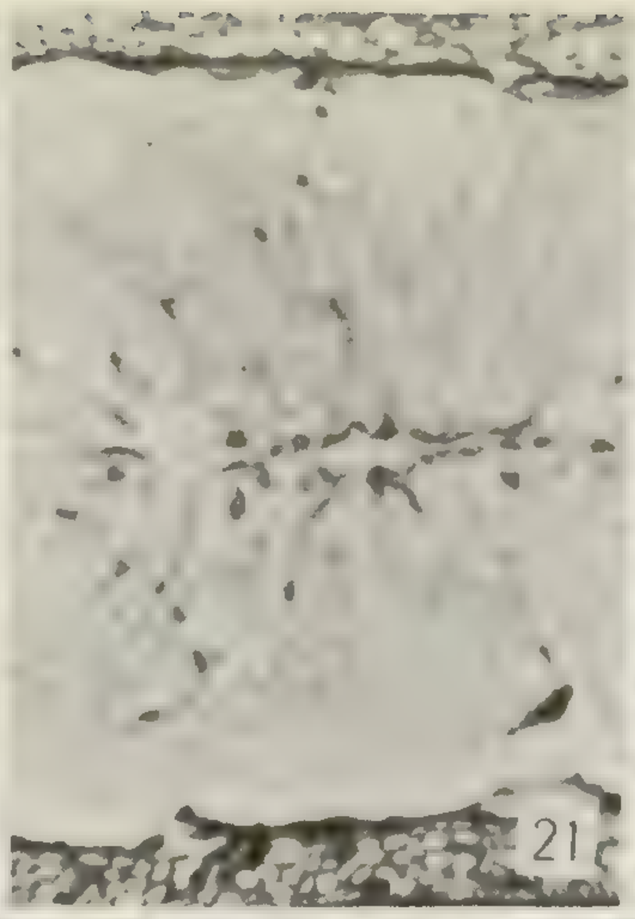
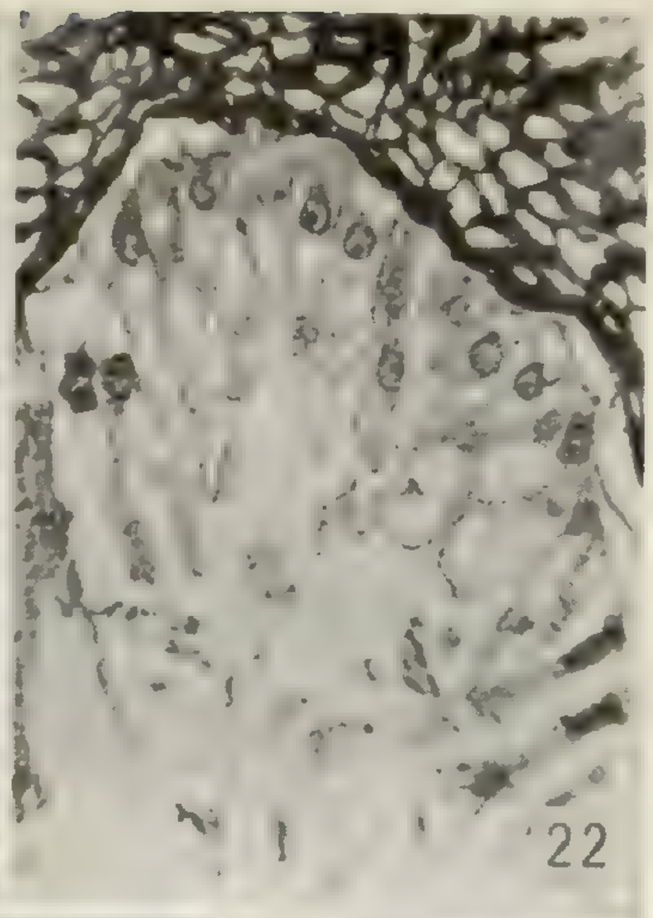
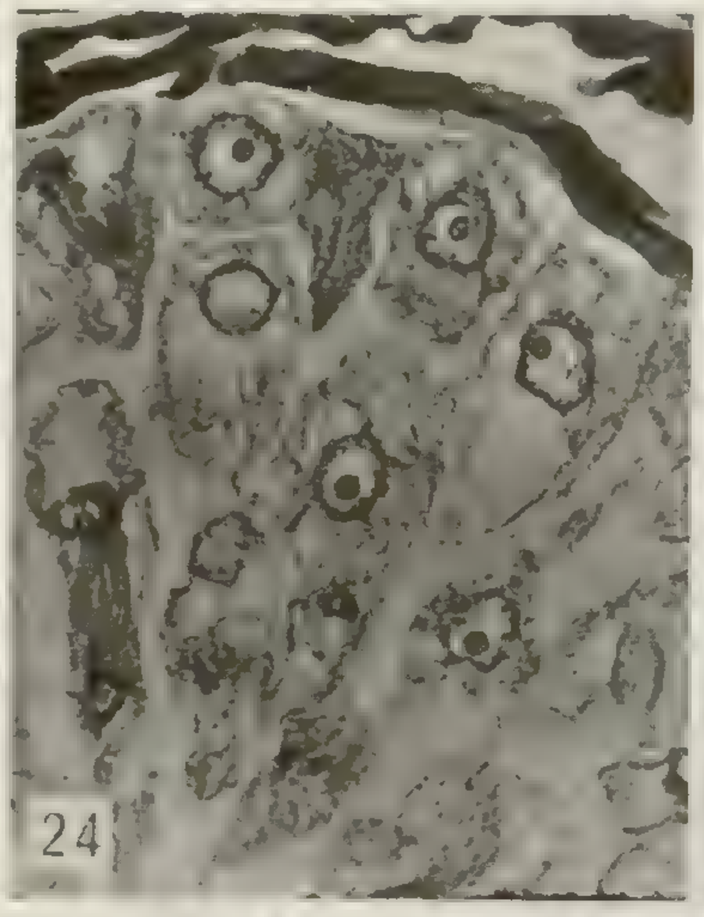
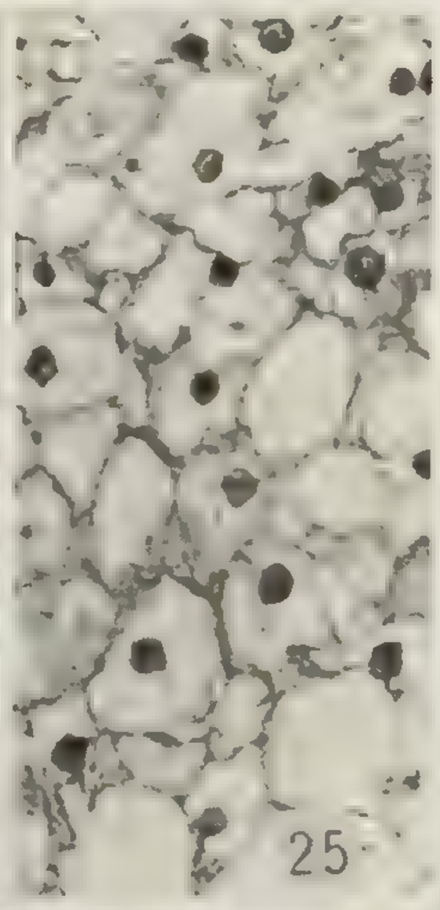
BURLINGAME on ARAUCARIA BRASILIENSIS

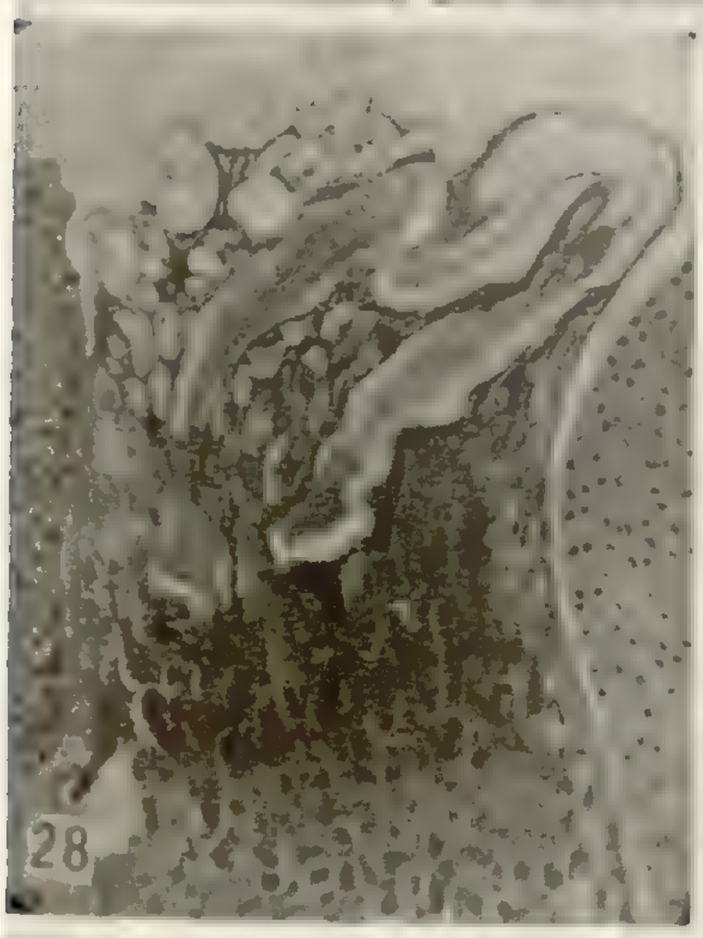


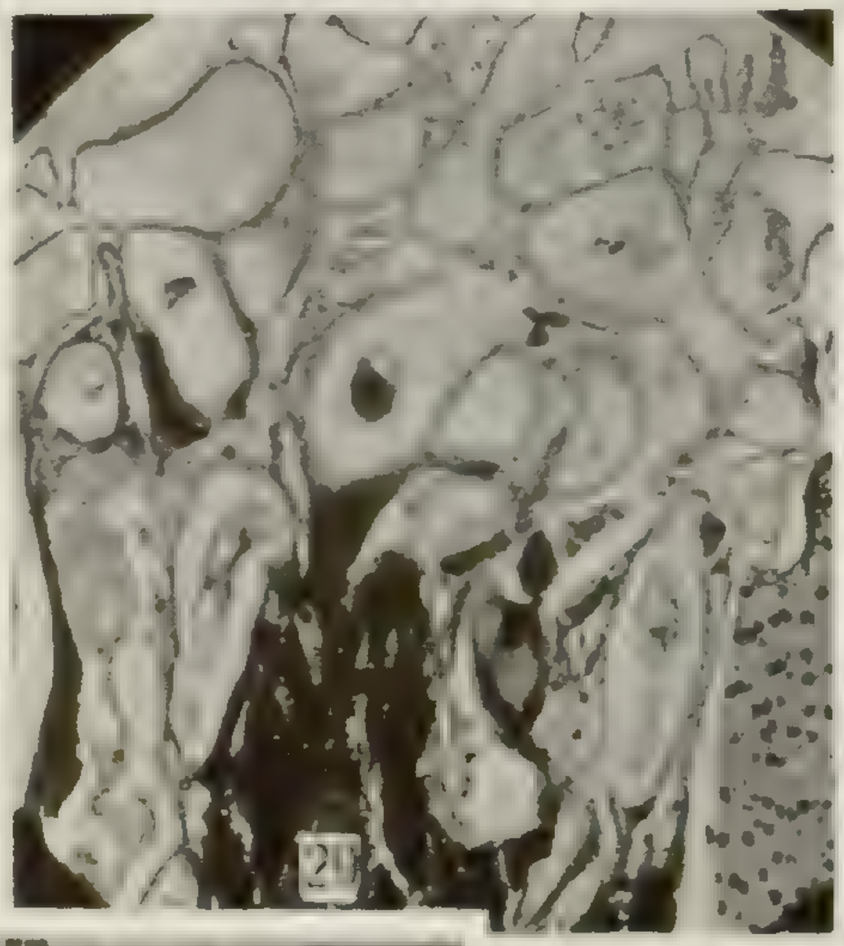


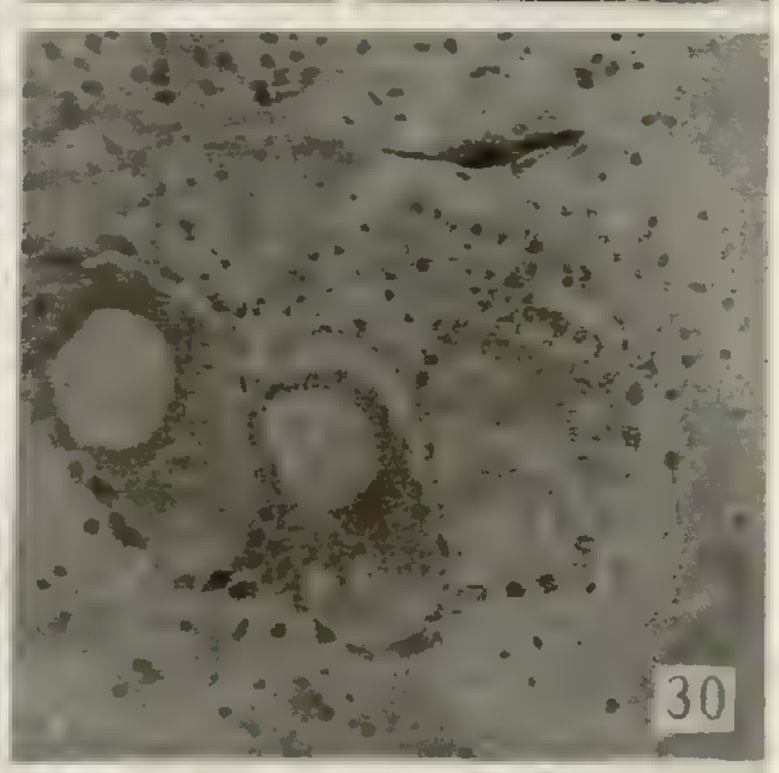


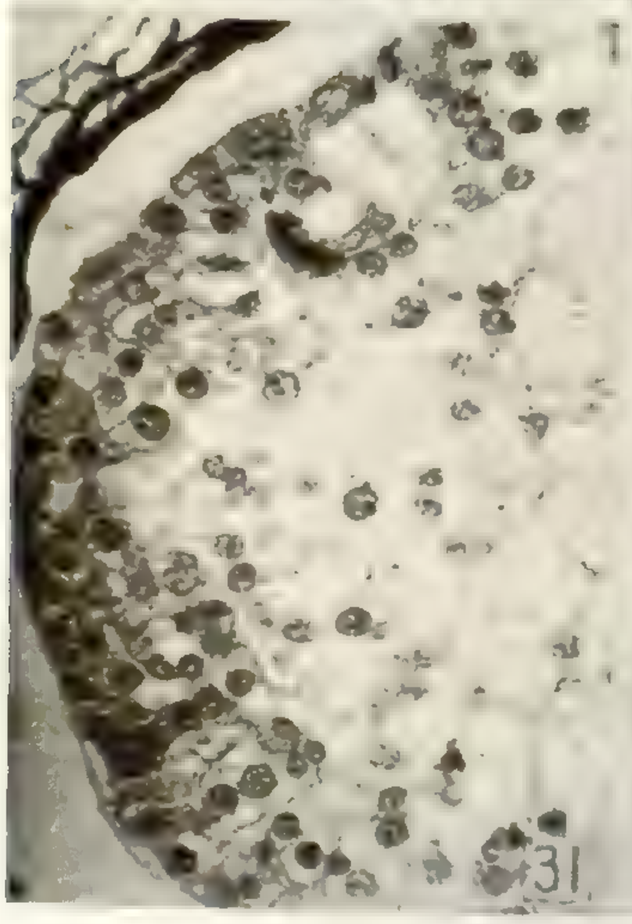


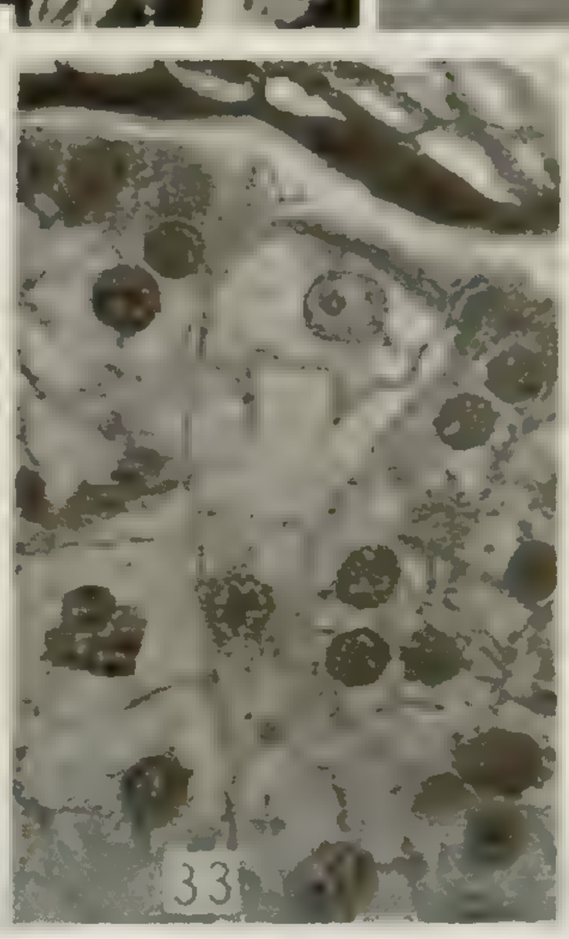


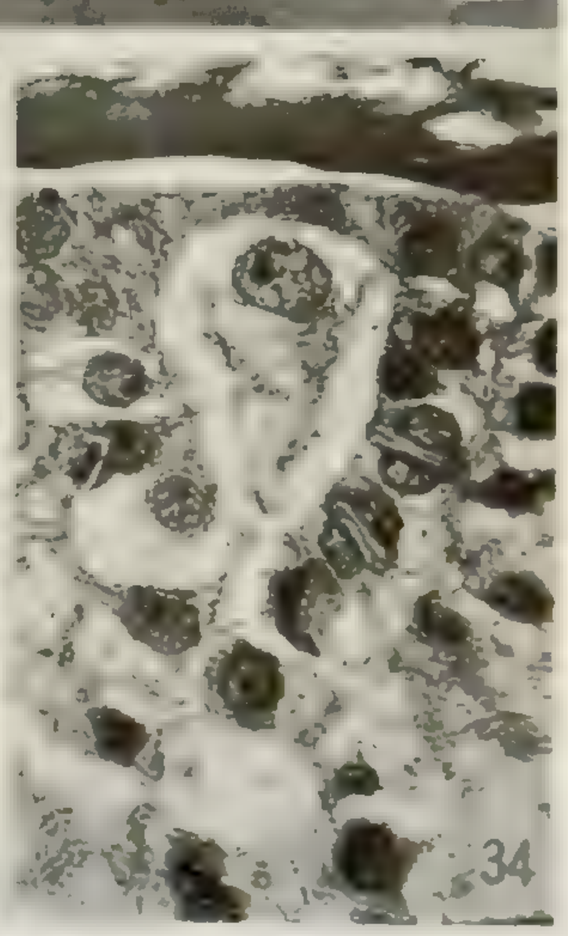




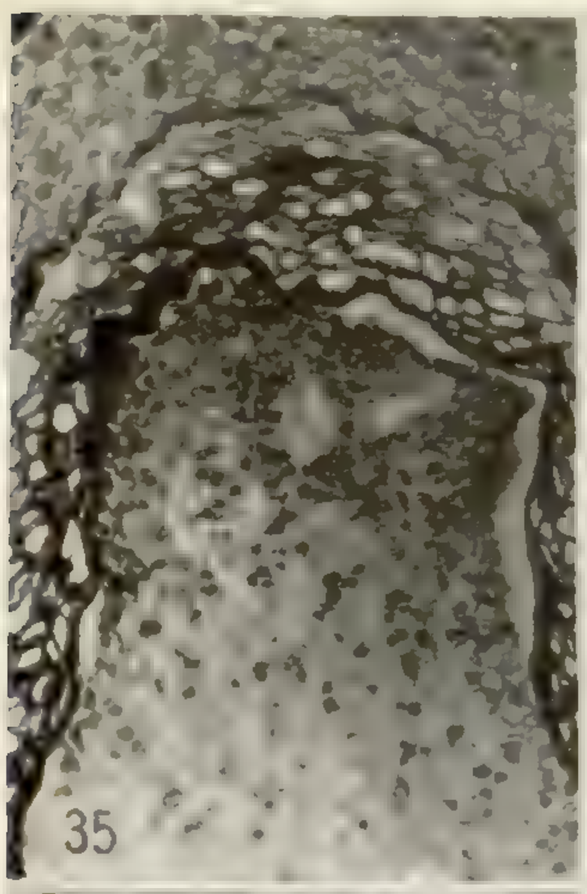


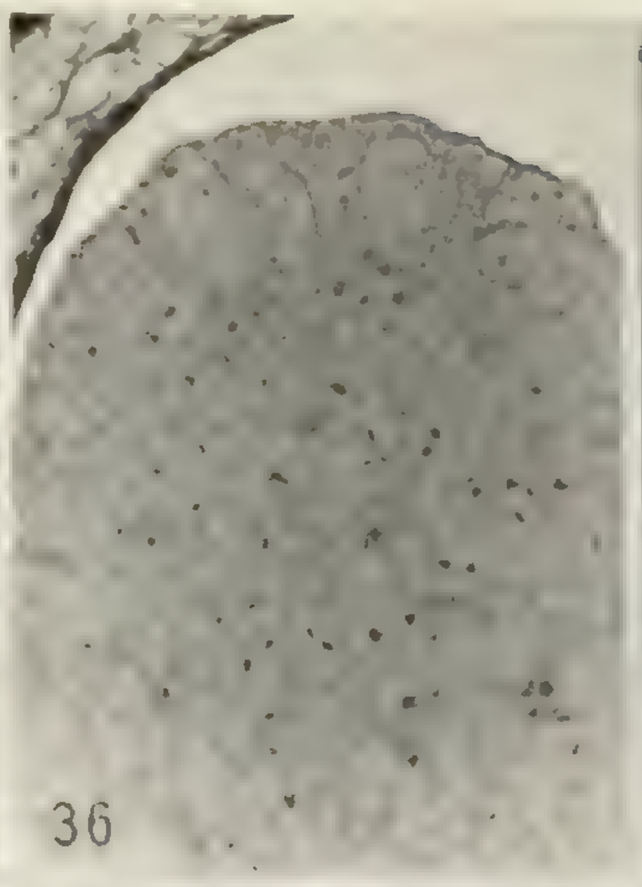


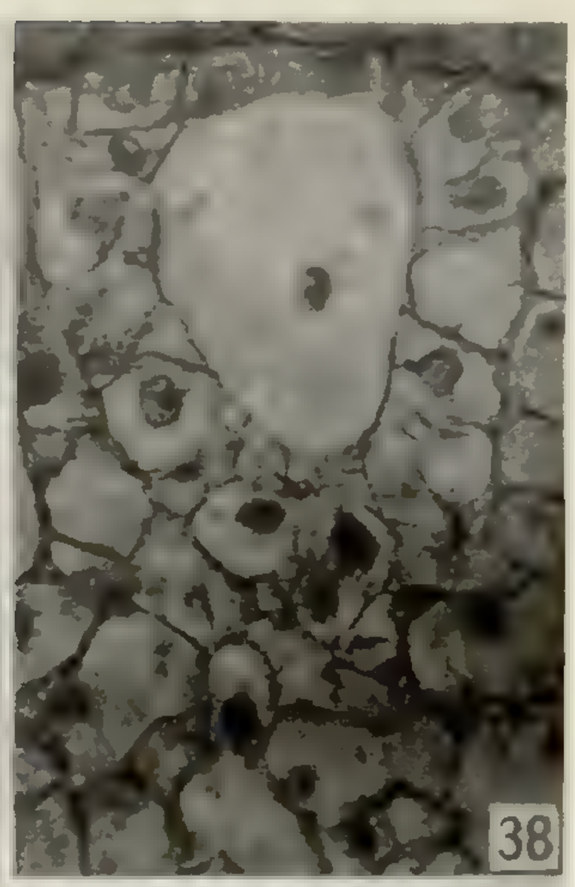


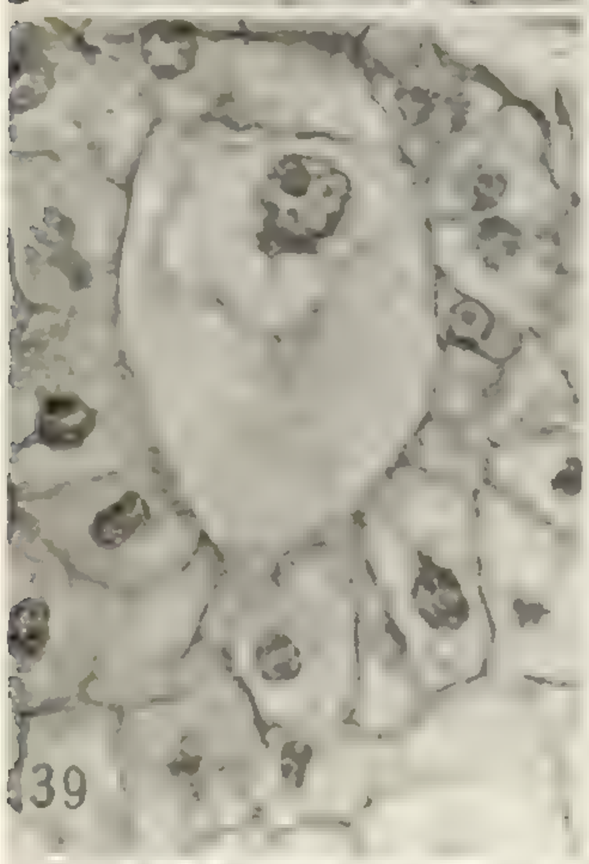


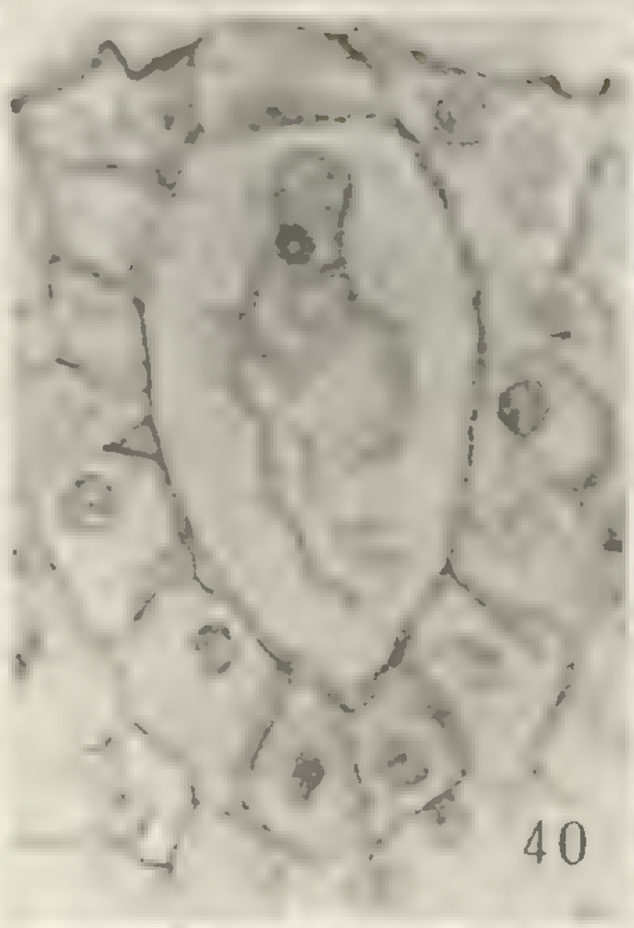
BURLINGAME on ARAUCARIA BRASILIENSIS

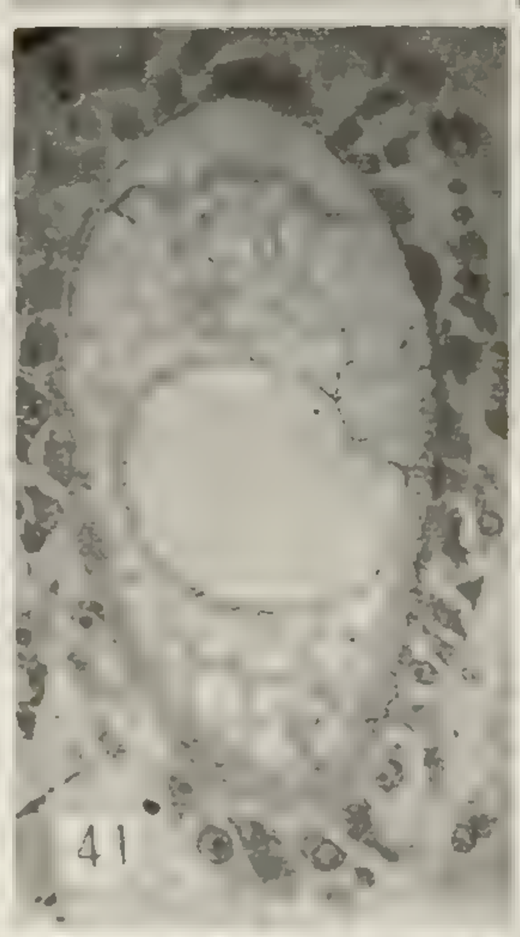


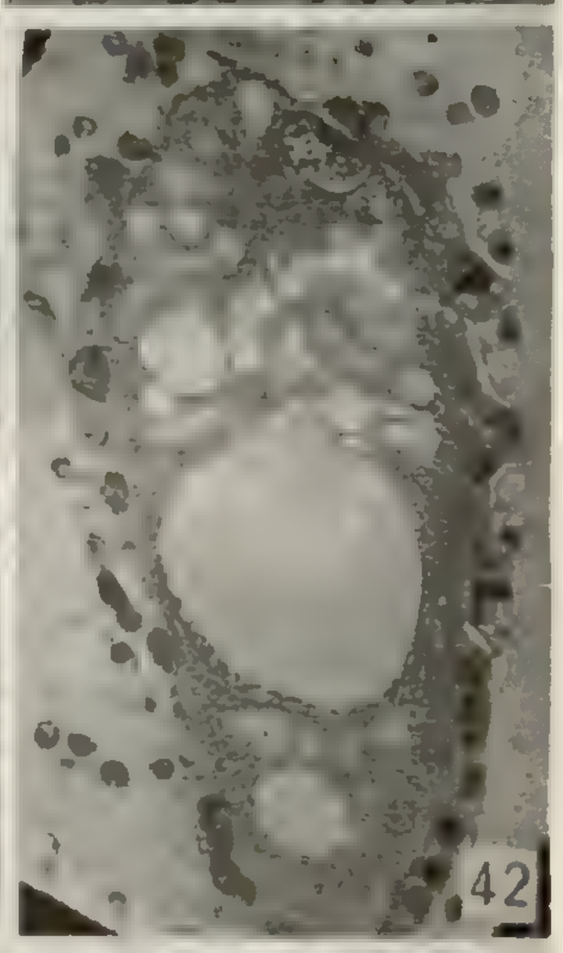


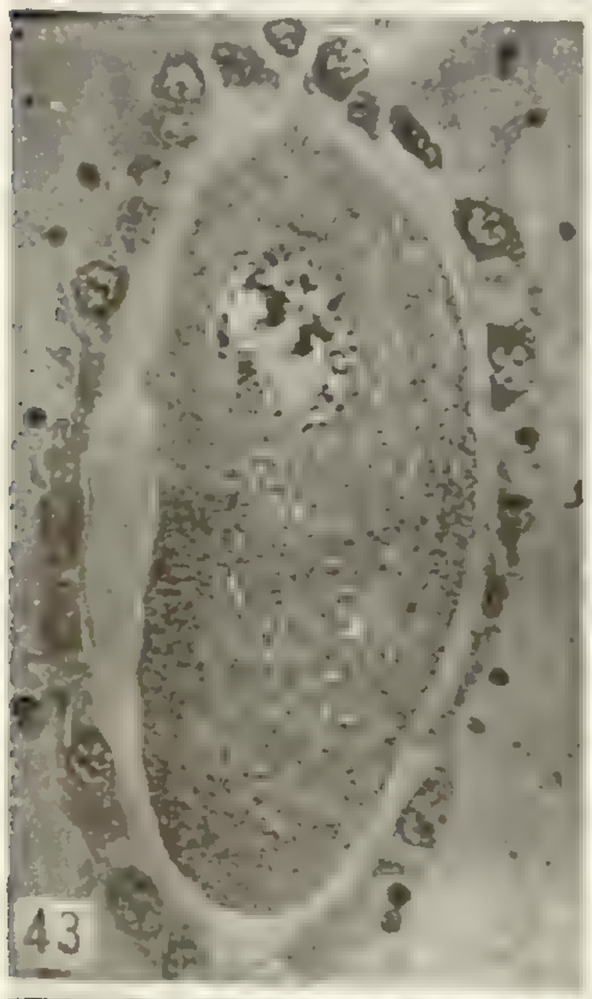


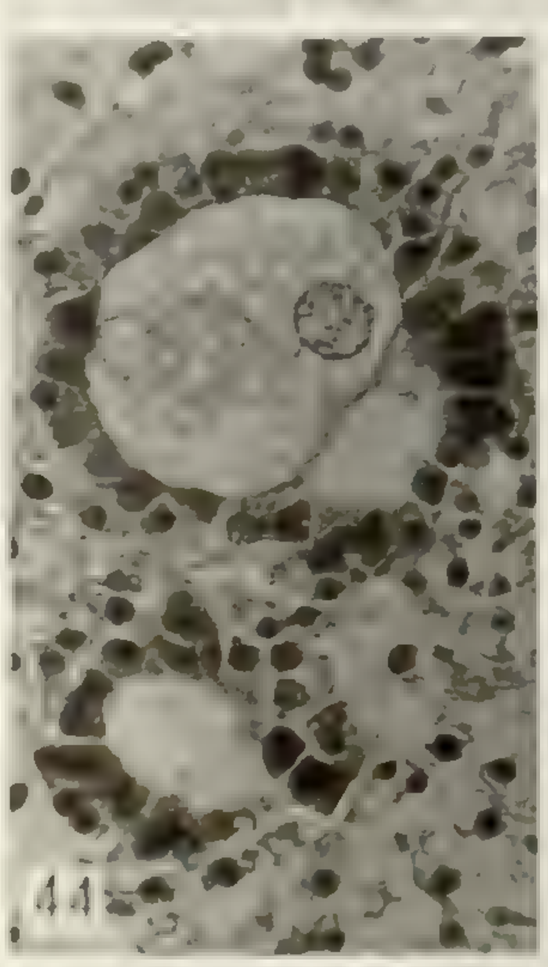


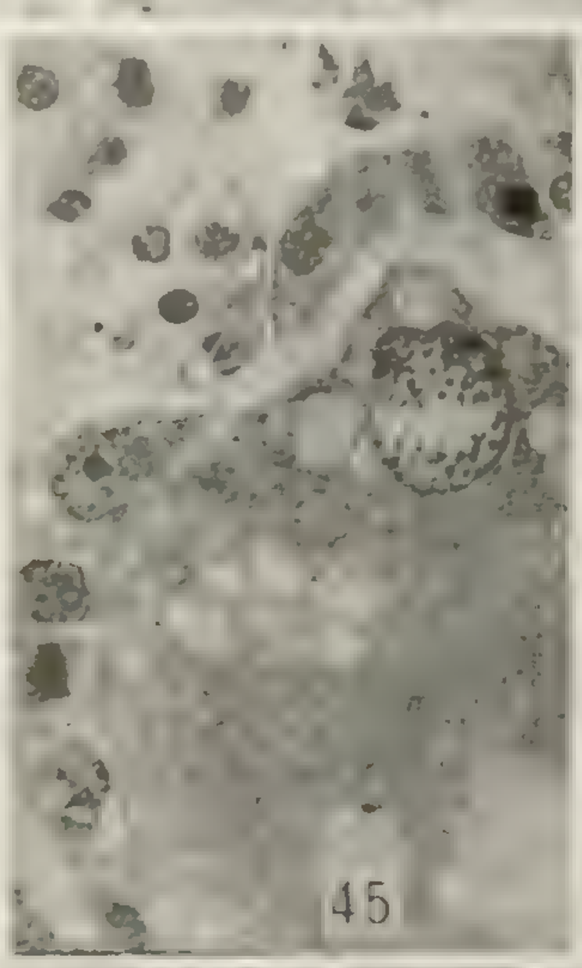


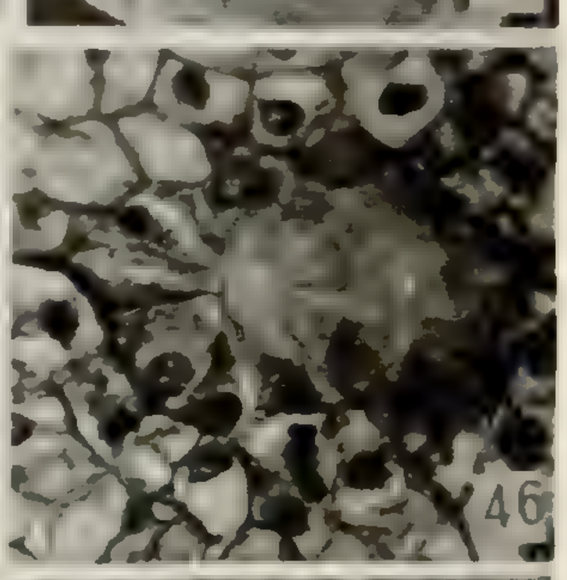


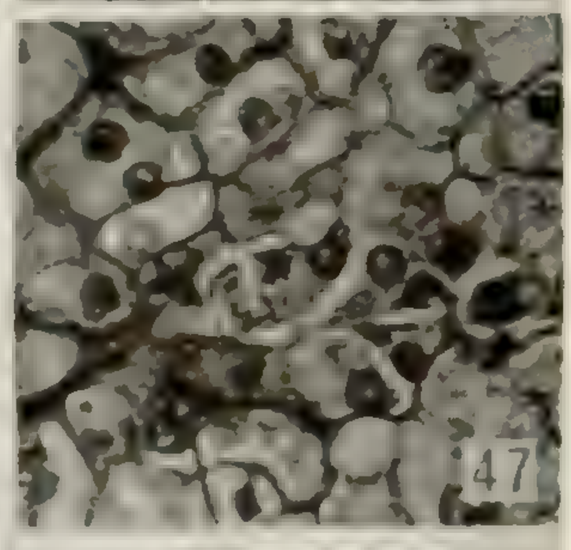


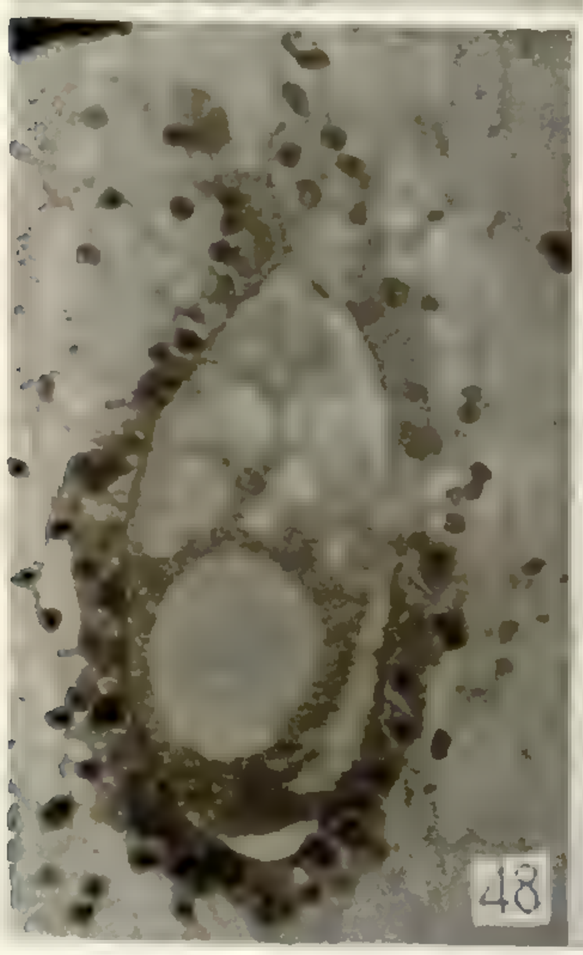


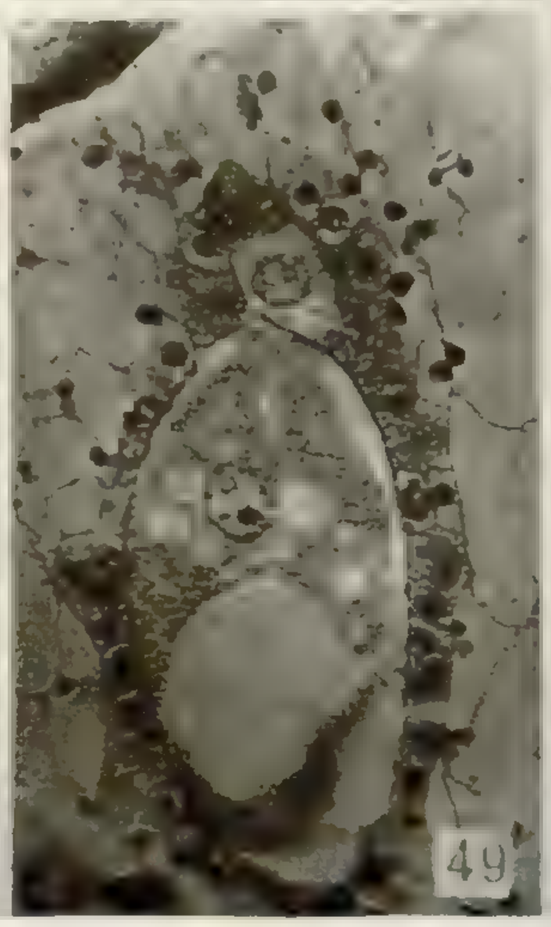


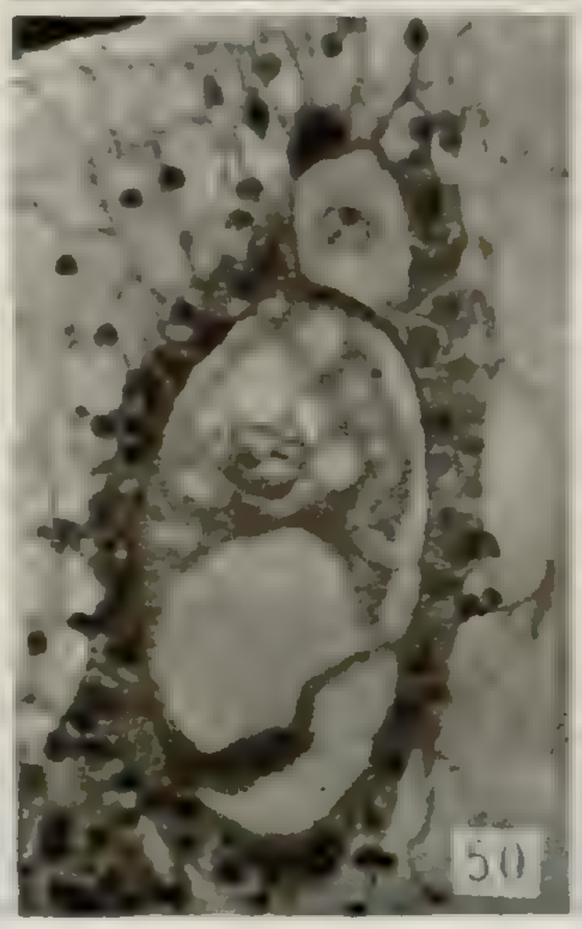


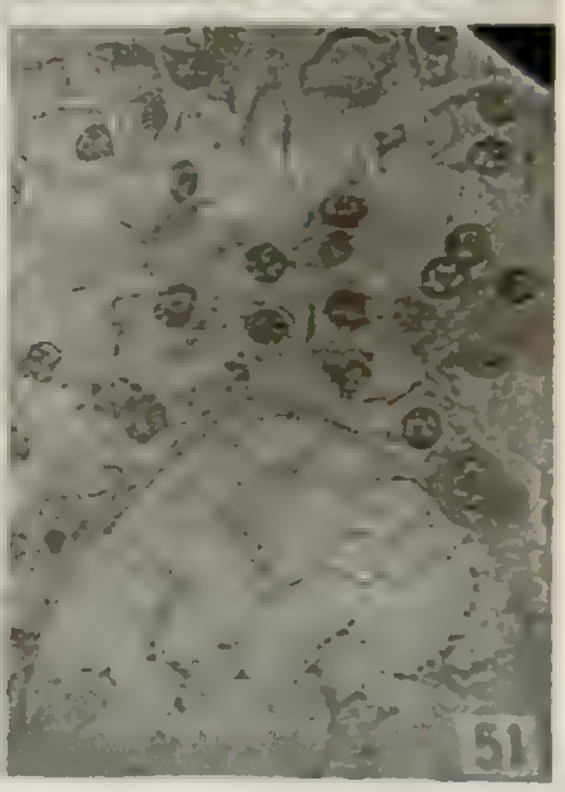












BURLINGAME on ARAUCARIA BRASILIENSIS

THE ORIGIN OF MONOCOTYLEDONY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 187

JOHN M. COULTER AND W. J. G. LAND

(WITH PLATES XXVIII AND XXIX AND TWO FIGURES)

The origin of Monocotyledons from the Archichlamydeae seems well enough established to need no discussion in this connection. The evidence of vascular anatomy, supported by the historical record, as well as by general morphological considerations, seems to be explicit. It remained to obtain evidence of the transition from dicotyledony to monocotyledony. This seemed to be a peculiarly difficult situation, for it appeared to involve much more than the number of cotyledons. The difference in number was disposed of in two ways, the monocotyledonous condition being said to have arisen either by a fusion of the two cotyledons or by a suppression of one of them. Each of these views can be supported by a considerable body of evidence, based upon vascular anatomy and upon many intermediate stages in fusion or in elimination. The real difficulty in the situation, however, appeared to be in the fact that in Monocotyledons the cotyledon is a terminal structure, and in Dicotyledons the cotyledons are lateral structures. How could the terminal cell of a filamentous proembryo which had been producing a stem tip change its function and persistently produce a cotyledon? Any comparison of the proembryos of *Capsella* and *Alisma*, the two accepted types of Dicotyledons and Monocotyledons, emphasizes this difficulty.

A clue to this problem was furnished by the seedlings of *Cyrtanthus*, a South African genus of Amaryllidaceae, which was investigated by Miss FARRELL,[1] a graduate student of this department. In accordance with this suggestion, seeds of numerous Monocotyledons were obtained from South Africa and Australia, These were germinated and an abundance of material obtained for study.

[1] FARRELL, MARGARET E., Ovary and embryo of *Cyrtanthus sanguineus*. BOT. GAZ. 57:428–436. *pl. 24. figs. 3.* 1914.

The first case to attract our attention was that of *Agapanthus umbellatus* L'Hér., one of the South African Liliaceae. Although ordinarily monocotyledonous, a seedling was found with two well developed cotyledons. This discovery led us to hope that it might be used in determining the relation between monocotyledony and dicotyledony. The two conditions shown by the seedlings of *Agapanthus* are illustrated in fig. 1. If the seedlings of the same species are thus indifferently monocotyledonous or dicotyledonous, there must be some evident relationship between the two conditions.

In the following account no attempt will be made to cite the literature of the subject. It is sufficiently well known to students of Angiosperms, and most of it is available in COULTER and CHAMBERLAIN'S *Morphology of Angiosperms*. When the remaining material that has become available is investigated, a more detailed account of the whole subject of the development of cotyledons will be given.

The structure of the monocotyledonous seedling of *Agapanthus*, a photograph of which is reproduced in fig. 1, is shown by the series of transverse sections given in figs. 2–13, and by their diagrammatic reconstruction in fig. 14. The section through the cotyledon and first leaf above the cotyledonary sheath (fig. 2) shows three vascular strands in the cotyledon, arranged in a triangle, with the xylem directed toward the center. If the middle strand had not been laid down, the two laterals would show the inverse orientation that has suggested that the monocotyledonous condition has arisen by a fusion of two cotyledons. The cotyledon above the sheath is cylindrical, but as the sheath is approached it begins to invest the first leaf (fig. 3). Soon the sheath becomes a complete ring in transverse section, which increases in thickness around the leaf as the transition region is approached, until, at the junction with the first leaf, the investing sheath becomes of almost uniform thickness (fig. 9), that is, the side of the sheath away from the cotyledon is as thick as the side which is continuous with the cotyledon. In all the many seedlings sectioned, the cotyledonary sheath is a simple ring in transverse section, without any infoldings.

The three strands retain their positions with reference to one another until just above the transition region, where they approach

one another, and their phloem strands become united (fig. 9). Farther down the three cotyledonary strands unite completely, and the phloem divides (fig. 10). The single bundle of the first leaf turns inward (figs. 9 and 10) and unites with the cotyledonary strands (fig. 11). The lateral bundles of the leaf are disregarded.

FIG. 1.—Monocotyledonous and dicotyledonous embryos of *Agapanthus umbellatus;* ×4.

since they appear late, and in early seedling stages are not connected with the main strand or with the cotyledonary plate. This union of strands, three from the cotyledon and one from the first leaf, forms a siphonostele (fig. 11), which is the "cotyledonary plate." The strand from the first leaf continues into the hypocotyl and becomes one of the poles of the root, and from the cotyledonary strands that enter into the structure of the cotyledonary plate, two other strands pass down the hypocotyl to form poles of the root,

which is therefore triarch. Farther down, the strand which has come from the first leaf forks, and the root becomes tetrarch. Older seedlings were not examined, but probably further forking of strands occurs, making the root eventually polyarch.

In thus describing the course of the vascular strands, the sequence given seems to be that of their formation, although we did not observe the procambial strands. If this be true, they are laid down first in the growing cotyledon and first leaf, become united later in the cotyledonary plate, which then gives rise to the root poles. This means that the vascular strands do not determine the development of the structures of the seedling, but that the primordia of cotyledon and first leaf start and the vascular strands are laid down in the growing organs. In other words, growing primordia determine the vascular strands rather than the reverse. If the cotyledonary sheath is very massive, vascular bundles may be laid down, and these may have no connection with other bundles. In the cotyledonary sheath of *Doryanthes Palmeri*, which is very thick, a vascular strand was found at a point opposite the massive cotyledon. This solitary strand had no connection with the cotyledonary plate, or with any other strand, beginning and ending blindly in the sheath. The inference seems evident that vascular strands are secondary structures, whose appearance is dependent upon the character of the primary structure, and therefore of no phylogenetic significance in the seedling.

It is noteworthy also that in the seedling of *Agapanthus* there is no stem primordium. In fact, the stem is a very belated organ, not having appeared in the seedlings under investigation. When and how a stem primordium is organized later was not seen. Certainly in the well developed seedlings of *Agapanthus* there is no stem primordium that gives rise to lateral leaf primordia. All of the meristematic tissue is involved in cotyledon and leaf-formation.

The structure of the dicotyledonous seedling of *Agapanthus* shown in fig. 1 is indicated by the series of transverse sections given in figs. 15–28, and by their diagrammatic reconstruction in fig. 29. The seedling is of the same age as the monocotyledonous one. The two cotyledons are the same length, but one is slightly thicker than the other (figs. 15 and 16). The cotyledonary sheath extends

farther upward on the side opposite the first leaf, giving the seedling a slight asymmetry (fig. 17), but soon the sheath becomes a symmetrical ring in transverse section (figs. 18 and 19). Each cotyledon has two lateral strands, as if the middle one present in the monocotyledonous seedling has not been laid down. The second leaf is also well developed, so that six strands approach the cotyledonary plate (figs. 21–23). These gradually converge, the phloem of the cotyledonary strands uniting with that of the first leaf (fig. 23), and lower down with that of the second leaf (figs. 24 and 25), but the xylem does not fuse until farther down (fig. 26). As in the monocotyledonous seedling, the cotyledonary plate is a siphonostele, and the strand of the first leaf continues directly as one of the poles of the triarch root, and lower down divides, resulting in a tetrarch root.

So far as the vascular strands are concerned, the two seedlings differ in the number laid down in the cotyledons. The larger single cotyledon contains three strands, while each of the smaller cotyledons of the dicotyledonous seedling contains two strands. The organization of the cotyledonary plate and of the root poles is the same in both cases.

It is obvious that this difference in the number of vascular strands does not determine the development of one or two cotyledons; the number of strands is simply a result of the development of one or two cotyledons; for vascular strands are differentiated in the tissues of a growing organ. It seems clear, therefore, that the appearance of one or two persistently growing points in the cotyledonary region of the proembryo determines the monocotyledonous or dicotyledonous condition; and that in *Agapanthus* the number of such growing points is variable.

In comparing the two seedlings, a suspicion might arise that the so-called dicotyledonous seedling, with its two leaves as well as its two cotyledons, is a case of the fusion of two embryos, or rather two produced by a single proembryo. This possibility has not been traced through in detail, but the vascular situation just described shows that the dicotyledonous seedling is merely a slight modification of the monocotyledonous one, and gives no suggestion of the "fusion" of two embryos.

It seems evident that the variability in the number of cotyledons could appear only in massive proembryos. The conception of monocotyledonous and dicotyledonous embryos has become a somewhat rigid one because it has been based upon such proembryos as those of *Alisma* and *Capsella* as types. It has become customary to regard these filamentous proembryos as primitive and typical of the two groups, and the massive proembryos that occur in both Monocotyledons and Dicotyledons as modifications. It is our belief that massive proembryos represent the primitive condition of proembryos in Angiosperms, and that only from such a proembryo could the monocotyledonous and dicotyledonous conditions have differentiated. After this differentiation, the difference has become relatively fixed by the reduction of proembryos to filaments. While massive proembryos occur in all the three great divisions of Angiosperms, they are notably present among the Ranales, from which the monocotyledonous branch seems to have arisen; and they are also retained by many of the Monocotyledons, notably the Arales and Liliales, and in these groups one may expect to find occasional dicotyledony or even polycotyledony.

The sequence of events in the development of the embryo of *Agapanthus* is as follows. As the massive proembryo enlarges, the root end elongates, thus remaining narrow and pointed; while the shoot end widens, becoming relatively broad and flattish. At this broad and flat end the peripheral cells remain more actively meristematic than do the central cells. It is this peripheral meristematic zone that is the cotyledonary zone. In this zone two more active points or primordia appear and begin to develop. Soon the whole zone is involved in more rapid growth, resulting in a ring or tube, but with the primordia still evident. The sequence of these two stages must be kept distinctly in mind, namely, (1) the appearance of primordia, and (2) zonation. The cotyledonary zone continues its growth until a tube of considerable length is developed, leaving the apex of the proembryo depressed. In the case of the dicotyledonous embryo of *Agapanthus* the two primordia on the rim of the tube continue to develop equally, the growth of the whole cotyledonary zone being shared equally by the two cotyledons. In the

case of the monocotyledonous embryos, the cells of one of the cotyledons gradually lose their meristematic activity, but those of the other one continue division, and as a result the so-called single cotyledon is developed, which is really the growth of the whole cotyledonary zone under the guidance of a single growing point. One cotyledon is not eliminated, but the whole growth is diverted into one cotyledon. Of course "tube" or "sheath" and "cotyledon" are all one structure, arising from the cotyledonary zone. As a result of the checked growth of one of the cotyledonary primordia, there soon develops the appearance of an "open sheath" and a "terminal" cotyledon. The developmental stages in this case, therefore, are first two cotyledons, and then, because of one-sided growth, one large cotyledon and one so small as to be easily overlooked.

The two primordia appearing upon the cotyledonary zone mark the places where vascular strands will be laid down. It would seem that the positions of vascular strands in such a case, therefore, are of no significance beyond indicating possibly the places where primordia have appeared, and also sometimes suggesting the number of primordia; but they cannot be used even for these purposes with absolute certainty.

It would be interesting to assemble the many cases of unequal cotyledons and note the variation in the checked growth of one of them, so that it ranges in appearance from a mere protuberance or ligule-like appendage, to a fairly well developed, but smaller, cotyledon. Such a series can be observed among the so-called pseudo-Monocotyledons. In such a series it would be noted also that if the checked growth of one of the cotyledons is very early, it will contain no vascular bundles, all the cotyledonary strands being laid down in the growing cotyledon. In many cases, as in *Cyrtanthus*, as growth is diverted from one cotyledon to the other, the procambium strands are also diverted toward the larger and later functioning cotyledon, and finally the strands unite. This phenomenon of fusing strands has been interpreted as an evidence of the fusion of cotyledons. In *Fourcroya Bedinghausii*, however, we found that the procambium strands of the primordium which represents a second cotyledon do not drift across and unite with the

strands of the single functioning cotyledon. In these cases, therefore, one cotyledon is developed whether opposing strands unite or not. In monocotyledony, therefore, as shown by *Agapanthus*, the number of vascular strands in the single cotyledon is likely to be greater than it would have been if both cotyledons had developed, for the cotyledon itself is larger. It is such cases that have suggested that monocotyledony has arisen by the suppression of one cotyledon. It is not so much the suppression of one cotyledon, as the growth of the whole cotyledonary zone to form a single cotyledon. In other words, in such a case a cotyledon is no more suppressed than are petals in Sympetalae.

A further examination of the proembryos of *Cyrtanthus sanguineus*, reported upon by Miss FARRELL (*loc. cit.*), shows that in this species four primordia may appear upon the cotyledonary zone, which for a time develop equally. Then the whole zone becomes involved in the more rapid growth, giving rise to the cotyledonary ring or sheath, but with the four growing points still prominent. In the next stage the cells of the ring between a pair of growing points on each side become more active, and the four original growing points begin to "grow together" in pairs, so that two cotyledons, each with two points, begin to appear. During this stage, which may be called figuratively a "fusion in pairs," the cotyledonary sheath still continues to elongate. Later on one of the two cotyledons begins to develop faster than the other, resulting in two unequal cotyledons, which are connected at base by the thick ring. Gradually the cells of the smaller cotyledon cease dividing, and, those of the other continuing to divide, the result is a seemingly single terminal cotyledon. The developmental stages in this case are four cotyledons, two cotyledons, and finally one large cotyledon, associated with another one so small as to escape ordinary observation. The suggestion here of the possibility of polycotyledony in *Cyrtanthus* is plain, and the explanation of the polycotyledonous condition among certain Gymnosperms seems evident.

In the current accounts of the embryogeny of *Sagittaria* and of other forms with filamentous proembryos, the development of the proembryo from the filamentous condition to the organization

of growing points has not been traced, and the general conclusion has been reached that the terminal cell forms the single cotyledon. An examination of the stages between the filamentous proembryo and the apparently terminal cotyledon has shown that this conclusion has been taken for granted rather than tested. It is obvious that a massive proembryo is formed before growing points appear. The terminal cell of the filamentous proembryo of *Sagittaria* develops a mass of tissue whose meristematic peripheral cells develop a ringlike cotyledonary sheath, which in the growing seedling is still recognizable as an extremely narrow ring opposite the functional cotyledon. Just within this sheath, opposite the functional cotyledon, is a plate of cells, one or two cells thick, occupying the site of the second cotyledon which started as a second growing point in the cotyledonary zone. This plate merges with the base of the sheath and the first leaf, and in one instance was observed to extend upward 160 μ, and ended in two points (fig. 30). This vestigial structure may be interpreted variously, but it seems most natural to regard it as a vestige of the second cotyledon. In *Sagittaria* and *Alisma* the "growing point" of the stem has been traced to a definite plate of cells beneath the cotyledon. This plate of cells, however, is not the stem primordium, but the primordium of the first leaf. The "notch" so characteristic of these embryos is developed by the checked growth of a cotyledon primordium on one side of the cotyledonary sheath, and at the base of this notch, which is really between two cotyledon primordia, the first leaf develops.

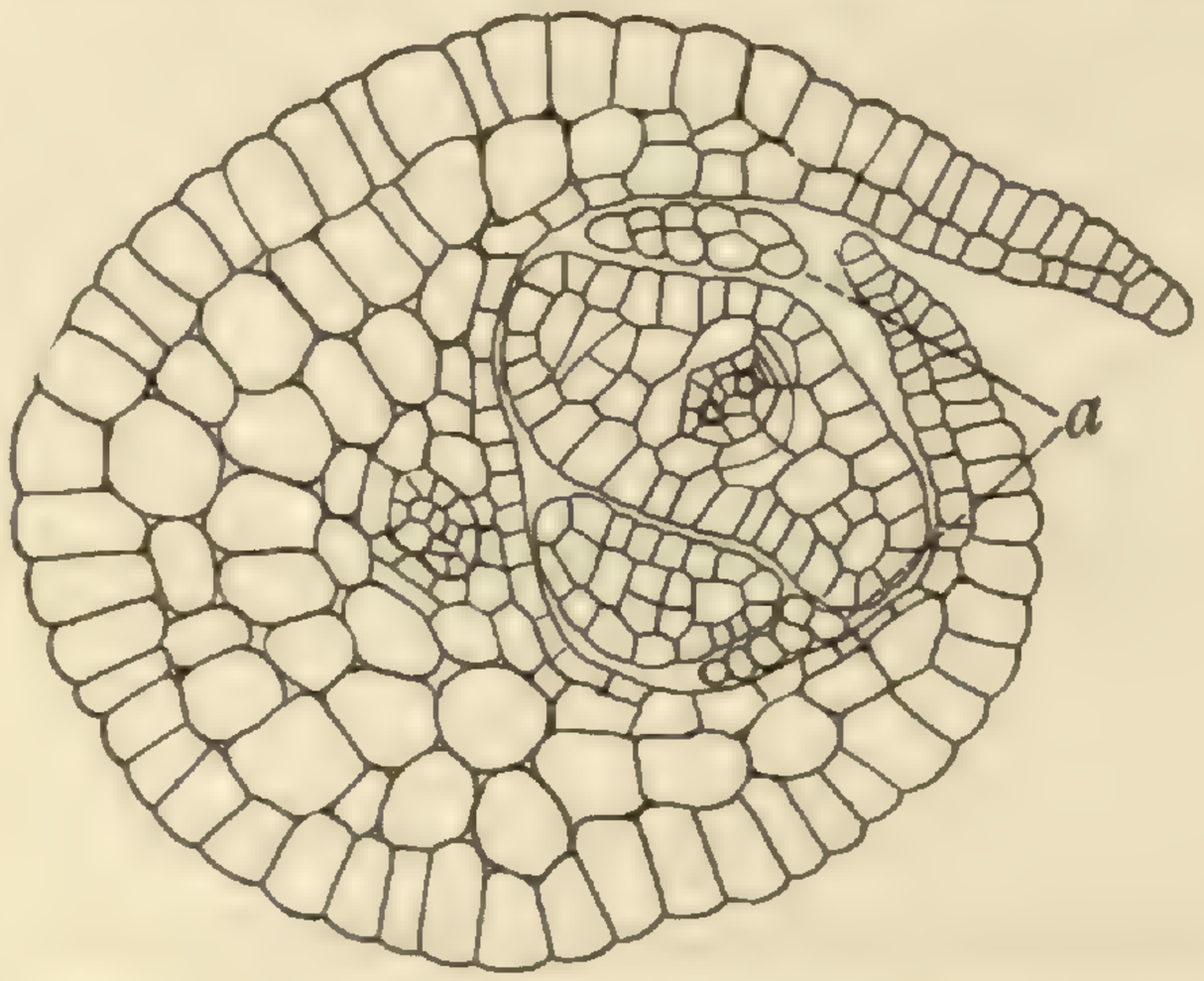


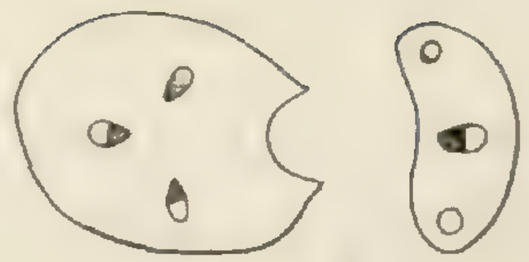
FIG. 30.—*Sagittaria variabilis:* transverse section immediately above the cotyledonary ring; *a*, rudiments of second cotyledon (?); ×150.

The conclusion is that in both Monocotyledons and Dicotyledons a peripheral cotyledonary zone gives rise to two or more

growing points or primordia, and that this is followed by a zonal development resulting in a cotyledonary ring or sheath of varying length. If both growing points continue to develop equally, the dicotyledonous condition is reached. If one of the growing points ceases to develop, the growth of the whole cotyledonary zone is associated with that of the other growing point, and the monocotyledonous condition is reached. In other words, monocotyledony is not the result of the fusion of two cotyledons, or of the suppression of one; but it is simply the continuation of one growing point on the cotyledonary ring, rather than a division of the growth between two growing points. In the same way polycotyledony is the appearance and continued development of more than two growing points on the cotyledonary ring. In fact, in *Cyrtanthus* four growing points appear at first, which under certain conditions might result in four cotyledons. The whole situation has its parallel in sympetalous corollas, in which there is zonal development associated with three, four, or five separate growing points, which, continuing development, are recognized as petals.

It follows that cotyledons are always lateral structures arising from a peripheral cotyledonary zone at the top of a more or less massive proembryo. This reduces cotyledony in general to a common basis in origin, the number of cotyledons being a secondary feature. The constancy in the number of cotyledons in a great group is no more to be wondered at than a similar constancy in the number of petals developed by the petaliferous zone.

The organization of the stem tip in the seedling is worthy of consideration. In the mature seedlings of *Agapanthus* there is no appearance of a stem tip; all of the meristematic tissue at the tip of the proembryo is involved in the peripheral cotyledonary apparatus and the centrally placed leaves. The stem can be regarded as existing only hypothetically in the siphonostelic cotyledonary plate. Later in the history of the seedling the central region of the embryo beneath the leaves elongates and the stem structure begins to appear. If the early leaves of a plant are very small, an organized stem tip appears earlier in the history of the embryo, but it is doubtful whether in Monocotyledons any stem structure appears until late in the history of the seedling. In fact,



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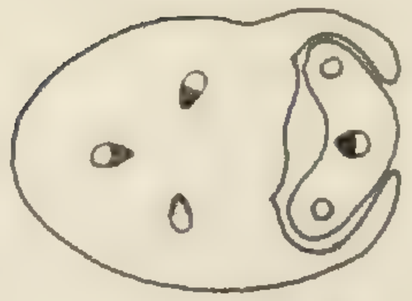

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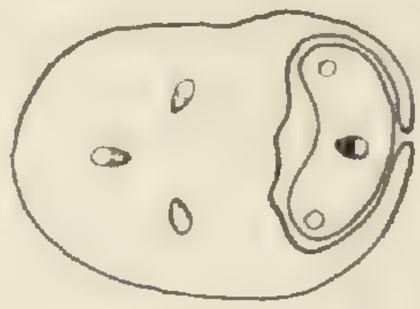

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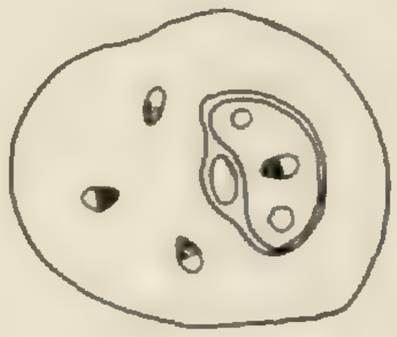

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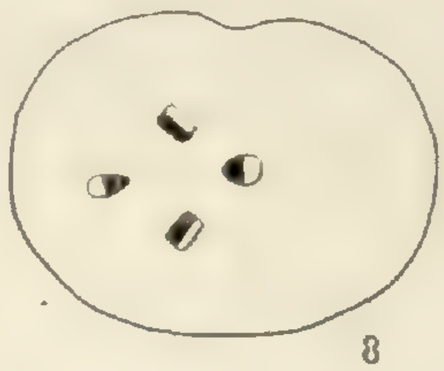

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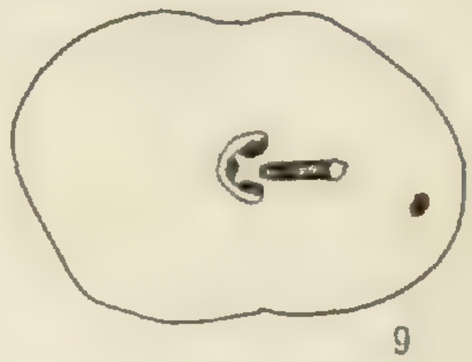

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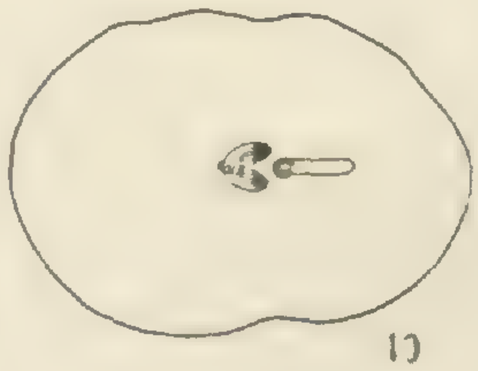

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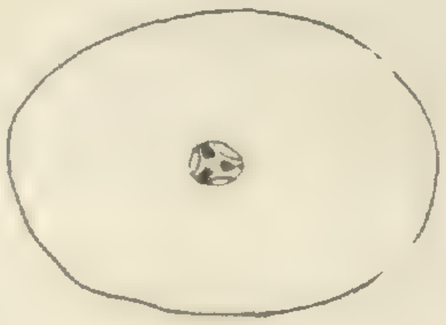

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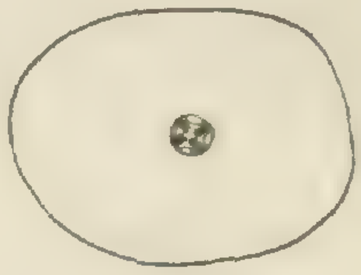

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W.J.G. Land, del.

COULTER and LAND on AGAPANTHUS

COULTER and LAND on AGAPANTHUS

the early and vigorous development of the first leaf in Monocotyledons is probably associated with checking the growth of the adjacent cotyledonary primordium.

Incidentally the late appearance of the stem as an organized structure probably enters into the explanation of the fact that the stem is the most advanced organ of the body in structure, as vascular anatomy has indicated repeatedly. In any event, the cotyledonary strands and first leaf strands organize the cotyledonary plate, which in turn gives rise to the root poles, and later determines the character of the stem cylinder.

The main thesis of this study of cotyledony, however, is to release it from its rigid morphological categories, by showing that the cotyledonary apparatus is always the same structure, arising in the same way, and varying only in the details of its final expression.

UNIVERSITY OF CHICAGO

EXPLANATION OF PLATES XXVIII AND XXIX

FIGS. 2–14.—Monocotyledonous embryo of *Agapanthus umbellatus:* figs. 2–13, transverse sections beginning just above the cotyledonary sheath and ending in the cotyledonary plate (the sequence of numbers indicates the sequence of sections); fig. 14, a diagrammatic reconstruction of the sections; ×14.

FIGS. 15–29.—Dicotyledonous embryo of *Agapanthus umbellatus:* figs. 15–28, transverse sections beginning just above the cotyledonary sheath and ending in the cotyledonary plate (the sequence of numbers indicates the sequence of sections); fig. 29, a diagrammatic reconstruction of the sections; ×14.

A METHOD OF CONTROLLING THE TEMPERATURE OF THE PARAFFIN BLOCK AND MICROTOME KNIFE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 188

W. J. G. LAND

(WITH TWO FIGURES)

The successful production of a continuous ribbon of paraffin depends chiefly on the temperature of the block in which the object is imbedded, and of the microtome knife. The hardness and size of the object may of course affect the ribbon, but if the object is small, not refractory, and completely infiltrated, the effect is practically negligible. If the temperature of the knife and block is made sufficiently low, very thin sections can be cut without unduly compressing the object, regardless of the melting point of the paraffin.

With paraffin of a given melting point there is a definite temperature at which the block and knife must be kept in order to produce a continuous ribbon of definite thickness without injurious compression of the sections. If the microtome is set for thinner sections without lowering the temperature of the knife and block, the sections are compressed more and more as they are made thinner, until a point is reached where the tissues are crushed out of all resemblance to their original condition. This crushing of the sections has in some instances resulted in erroneous interpretation of structures even by otherwise competent investigators. Again, if the microtome is set for thicker sections and the temperature of the knife and block is not correspondingly raised, excellent sections result, but their edges refuse to weld and they come away singly, a source of much trouble when an absolutely unbroken series is required. If the thickness is further increased, the sections come away rolled so tightly that in some instances they cannot be unrolled even by floating on warm water.

In practice, when thick sections (10–20 μ) are wanted, the object is imbedded in paraffin melting at 45–52° C. If thinner

sections are required (3–5 μ), paraffin melting at 58–62° C. is necessary. Even when the harder paraffin is used, in order to cut sections 2–4 μ thick, at ordinary room temperature, the knife and block must be cooled. If they are not cooled below the room temperature, which in winter is usually about 20–22° C., the sections are hopelessly crushed. In summer it becomes increasingly difficult to make thin sections.

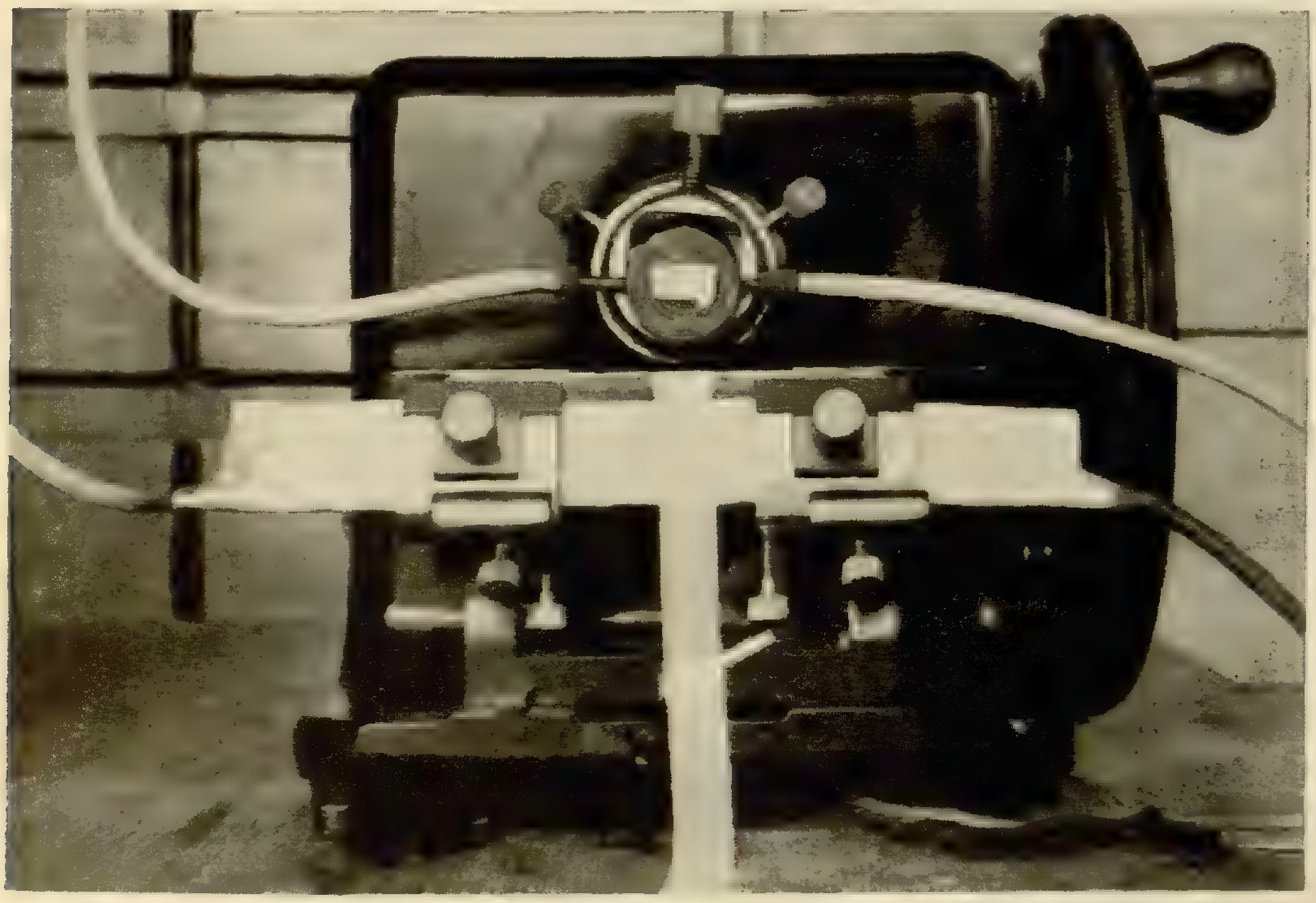

FIG. 1.—Apparatus for temperature control, showing object holder and cooling trough in place.

Many delicate plant tissues, notably certain liverworts, can be imbedded in paraffin melting at 45–52° C. with perceptibly little injury, but when the same material is imbedded at a temperature of 58–62° C. serious injury may result. For example, a tropical species of *Notothylas*, which at 52° showed little shrinkage, at 62° shrunk to nearly half the original thickness of the thallus. In addition to the action of heat, the effect of the large coefficient of expansion of paraffin (0.00027854) becomes marked when the harder paraffin is used. It follows that by using paraffin of a low melting point, the effect of heat and of the coefficient of expansion can be minimized.

In this laboratory the practice has been to cool the knife and block with lumps of ice. This method, while in the main giving good results, is unsatisfactory for the finest sectioning. Water usually gets on the ribbon and does damage; also great care is necessary to keep the microtome from rusting.

The various difficulties were in a large measure overcome as

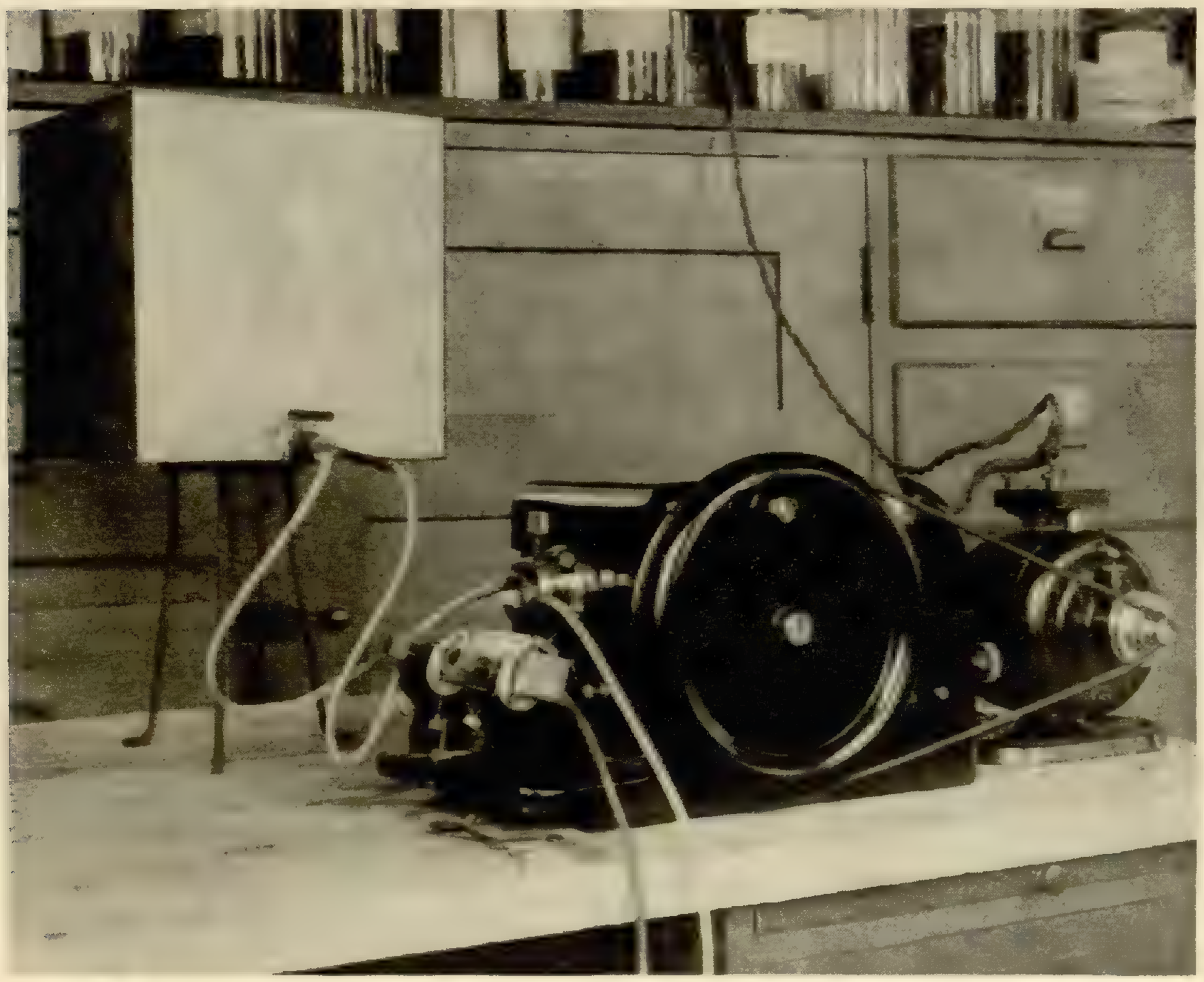

FIG. 2.—Apparatus for temperature control, showing all attachments

follows: The face of the ordinary circular metal object holder, which comes with all microtomes of the better class, was turned out to form a cup. A plate of thin brass was soldered over the cup, making a new face for the object holder, and turned down to the original diameter of the disk. Two holes were drilled and tapped at opposite sides of the disk and two short tubes for attaching small rubber tubes were screwed in. Fig. 1 shows this disk in the clamp of the microtome.

For controlling the temperature of the knife, a trough of thin metal—tin, brass, or copper—about 20 cm. long and about 2 cm.

deep was made to take in the knife and to permit about 1 cm. of the knife to project. A nipple at each end of the trough serves to attach small rubber tubes (fig. 1). The ends of the trough should be soldered to prevent leaking on the microtome. Any microtome knife having a detachable handle can be used. A flexible safety razor blade may be used, provided the heads of the clamping screws are flush with the sides of the blade holder.

A tank provided with a stopcock and Y-tube is placed above the microtome at a height which will insure a good flow of water. One tube of the "Y" is attached to a tube of the object holder by a rubber tube of small caliber, the other to the nipple of the cooling trough. Tubes lead from the holder and from the trough to a waste receptacle. The tank is filled with water of the proper temperature for the required thickness of ribbon. The block and knife reach the proper temperature for cutting soon after the water is turned on. The entire apparatus is shown in fig. 2.

The apparatus was designed primarily for cutting very thin sections (2–4 μ) of liverworts which had to be imbedded in soft paraffin, but it has been found useful when very thick sections (20–50 μ) are wanted. In cutting the latter, the knife trough is detached, and the tank filled with warm water which is allowed to flow through the object holder only. The temperature of water necessary for cutting sections of various thicknesses is easily determined by experiment.

University of Chicago

BRIEFER ARTICLES

SUCCESSFUL ARTIFICIAL CULTURES OF CLITOCYBE ILLUDENS AND ARMILLARIA MELLEA

(WITH THREE FIGURES)

During the course of some culture work with the wood-destroying polypores in the fall of 1913, it was found of interest to try out similar methods with an agaric form. Spores were obtained from a fungus that at the time was identified as *Clitocybe illudens*, and from it dilution cultures were made on a beef-malt-agar medium. The spores were found to germinate readily, and in the course of three or four days numerous separate colonies appeared on the agar surface. No evidence of contamination being visible, separate colonies were transferred to sterile culture tubes of the same medium on November 15. Vigorous growth took place and the tubes soon displayed thick felts of a brownish-white mycelium.

FIG. 1

Early in December a small dark brown area was noticed in one of the cultures, which soon gave rise to several dark brown finger-like papillae. These continued to elongate, and lighter colored, somewhat more slender regions appeared at their tips. These now enlarged rapidly and soon

took on the "button" form of the young fruiting agaric. On December 26, the accompanying photograph (fig. 1) was taken, and four days later the pilei had opened to the mature condition shown in the second photograph (fig. 2). As will be seen, the fruit bodies developed in a quite normal manner, and, except for their size and the somewhat "recurved" condition of the pilei, appeared to be quite normal. Fruit bodies examined with the microscope were seen to be sporulating profusely, and the spores were found to be quite normal as to color, shape, and size for this form. Upon being transferred to the beef-malt-agar medium, the spores germinated quite as readily as those from the original fruit bodies. Cultures are at present being maintained with the hope of obtaining a second fruiting generation, but as yet (March 14, 1914) no indications of the formation of fruiting bodies of the second generation have been observed.[1]

FIG. 2

The fact that the formation of normal fruit bodies on a synthetic medium is somewhat rare led me to consider what possible conditions may have effected this result. Cultures were found to fruit in either light or darkness, and so the presence or absence of light as a factor seemed to be eliminated, although it must be said that the first stages were always initiated in the dark. All cultures from the same fruit body made on one particular lot of medium, which had been slightly

[1] Since the preparation of this article, numerous fruiting bodies of the second spore generation have been obtained, showing such striking variations in form from the original parent that it has been thought best to discuss this phase in a future paper.

scorched in preparation, either fruited or produced abortive fruit bodies, while no fruiting was observed in other cultures. The scorched condition of the medium would of course give rise to substances not generally present in the culture medium, and it is suggested that this condition of nutrition may have been the determining factor. It is also of interest to note that the fruit bodies from which the spores were obtained were frozen solid when collected, which did not appear to injure the viability of the spores.

FIG. 3

Cultures of another very interesting agaric were obtained from the pathologist of the Forest Products Laboratory at Madison, Wisconsin, namely *Armillaria mellea* Vahl. This species forms a whitish mycelium which soon turns to a dark brown. The interesting feature, however, is the formation of the so-called "rhizomorphs." These are described as appearing in nature as shining black strands often resembling the roots of the host. They appear soon after inoculation on agar cultures, ramifying throughout the substratum. Here, however, they are of a shining light gray color, and are flat and ribbon-like, often branching dichotomously (fig. 3). Upon pentrating to a free surface these rhizomorphs immediately give rise to the ordinary vegetative mycelium.—V. H. YOUNG, *University of Wisconsin, Madison.*

THE AMOUNT OF BARE GROUND IN SOME MOUNTAIN GRASSLANDS

In July 1911 the writer staked out a series of 19 quadrats for study of the grassland of a mountain park at Tolland, Colorado. In that year collections were begun and censuses of some of the quadrats made. The plan was adopted of estimating at intervals the percentage composi-

tion of each quadrat. Early in 1912 more detailed work was undertaken, all findings being recorded on catalogue cards. Each time a quadrat was examined an e timate[2] was made of the percentage of bare ground. Since there seem to be no published records of such studies in our mountains, it has occurred to the writer that a brief note on the subject would be of interest.

The mountain park under consideration is a small glaciated valley in the Rocky Mountains at an altitude of about 9000 feet. Thc climate is cold, the July mean being about 56° F. Most of the park has a coarse soil of decomposed granite. Upon this is a xerophytic grassland vegetation. In certain places, however, particularly at points along the margins of creeks or ponds and in glacial sinks, a mesophytic grassland develops. This may be spoken of as "meadow." Here the soil has a considerable amount of humus derived from washings of adjacent slopes. Of the 19 quadrats, 17 are in dry grassland and 2 in meadow.

The data presented (tables I and II) are arranged according to time of year. Although the observations were made in two seasons, no doubt they show normal conditions, since both years had about the usual precipitation and were not greatly different in temperatures.

TABLE I

PERCENTAGE OF BARE GROUND IN DRY GRASSLAND

Quadrat	May 30, 1912	June 12, 1913	June 28, 1913	July 12, 1913	July 26, 1913	Sept. 1, 1912
I	75	60	.....	.....	30	36
II	60	49	.....	.....	33	53
III	63	42	.....	.....	50	46
IV	50	20	.....	.....	13	20
V	60	55	.....	.....	20	40
VI	60	50	.....	.....	28	33
VII	50	38	.....	.....	20	28
VIII	70	55	25	30	45	30
IX	64	30	25	15	20	30
X	63	25	20	20	10	20
XI	80	75	50	32	50	40
XII	50	.....	20	.....	15	25
XII (*a*)	60	40	20	10	15	20
XIII	50	.....	10	.....	20	25
XIV	60	.....	20	25	.....	.....
XV	50	.....	20	.....	25	50
XVI	60	.....	10	.....	.....	30
Average	60	45	22	22	26	33

[2] It may be well to state that a new library card was taken each time for every quadrat study. The data were not tabulated or even looked at until the close of 1913, lest the judgment of the observer at any time might be influenced by earlier records.

The figures of 60 per cent bare ground for May 30 in table I should not be understood to mean that the other 40 per cent is fresh growth. Indeed, there is almost nothing green at that time of year. The vegetation cover is made up of the dry grasses, sedges, and flowering herbs of the previous summer. By July 1, however, the new growth has reached its maximum. This rapid development is necessitated by the shortness of the growing season. Only such plants as can mature quickly are able to exist under the rigorous climatic conditions.

TABLE II

PERCENTAGE OF BARE GROUND IN MEADOW GRASSLAND

Quadrat	May 30, 1912	June 12, 1913	June 28, 1913	July 12, 1913	July 26, 1913	Sept. 1, 1912
XI (*a*)	45	20	0	0	0	10
XIII (*a*)	25	.....	20	10	10	10
Average	35	20	10	5	5	10

In the dry grassland, as will be seen from table I, there is a considerable proportion of bare ground, even at the height of the season in early July. The meadow association, on the other hand, shows little or no vacant space at that time. The differences depend chiefly on the texture of the soil, the finer grained material of the meadow holding more moisture and permitting more luxuriant growth.

It is interesting to note that when introduced weeds get a foothold in the region they do not become established in the open association of dry grassland, but in the already closely grown meadow.—FRANCIS RAMALEY, *University of Colorado, Boulder, Colo.*

THE OXIDASES OF ACID TISSUES

According to recent theories, the oxidases play an essential part in respiration. If these theories are correct, it follows that oxidases must be present in all organisms. But a serious difficulty is encountered in the fact that many plants have been reported to be free from oxidases. If this is really the case, it would seem that these theories must be abandoned, or at least greatly modified.

It appeared to the writer that in view of the importance of the subject a fresh investigation was needed. From the results of the writer's experiments it is evident that in many cases the reported absence of oxidases is to be ascribed to faulty methods of investigation. The

method which has been almost exclusively used is to express the juice or to make watery extracts of the ground or minced tissue. The ability of these extracts to accelerate the oxidation of such reagents as gum guaiac, α-napthol, hydrochinone, etc., has been taken as an indication of the presence of oxidases in the respective tissues.

The extracts which appear to be without oxidases when tested in this manner have, in the majority of cases, a distinct acid reaction. This fact was noted by CLARK[3] in summarizing the results of his own experiments. He concluded that extracts having an acidity such that 10 cc. required 8 cc. 0.1 N, KOH to neutralize to phenolphthalein were without oxidases.

The writer, proceeding in a similar manner with a considerable number of acid tissues, including several not previously tested, obtained results comparable with those reported by CLARK. He then made a series of special tests on *Citrus* fruits, probably the most acid of tissues, using a different method of examination, which seems to throw light on the general condition in acid tissues. Lemons, oranges, grape fruit, and kumquats were tried. In these fruits the juice is contained in long and enlarged sacs attached to the inside of the carpel walls. From this point of attachment they extend into the carpel, giving it the familiar honeycomb-like appearance. These sacs may be readily separated from the carpels and from one another without injuring their skin, which consists of 2–10 layers of cells. In this condition, when placed in a solution of gum guaiac, α-napthol, sodium selenite, or similar reagents, along with hydrogen peroxide, the surface of the hairs soon becomes covered with the colored compound resulting from the oxidation of the reagent. This indicates the presence of oxidases (or, according to the terminology of BACH and CHODAT, peroxidases, since hydrogen peroxide was required) in the cells of the sacs which contain the juice.

It is thus evident that *Citrus* fruits have normal oxidases in their acid tissues. It is also evident that these oxidases are protected in some manner from the action of the acid which at this concentration effectually inhibits the action of oxidases. It seems to the writer that this protection may be afforded by a semipermeable surface (the plasma membrane or cell walls similar to the cell walls of barley seed, which are impermeable to acid) through which the acid is unable to pass. When the tissue is ground, previous to pressing out the juice, the structure which separates the acid from the ferment is destroyed, so that the action of the latter is inhibited. That these membranes are not normally

[3] CLARK, Torreya 11:101. 1911.

permeable to acid is shown by the fact that seeds[4] of lemons (which are separated from the acid by the walls of the sacs) frequently germinate while still in the carpels, though they will not germinate in lemon juice several times diluted.

It seems probable that this condition is a general one in acid tissues. The acid and ferment are separated in the tissue probably in a variety of ways, but the grinding destroys the separating surface, bringing acid and ferment in contact and inhibiting the action of the latter.

The general effects of acids and alkalies on oxidase ferments are now under investigation and will be reported on later.—G. B. REED, *Laboratory of Plant Physiology, Harvard University*.

THE TYPE SPECIES OF DANTHONIA

In a recent paper,[5] Professor A. S. HITCHCOCK attempts to show that the type species of *Danthonia* DC. is *Avena spicata* L. instead of *Festuca decumbens* L. If his arguments are to stand, stability in nomenclature will be an impossibility, for they are based purely on personal opinion. If a case in which there is doubt as to the type of a genus is to be decided on an interpretation of what the author may have thought most representative, it will never be disposed of, for this decision may vary with each succeeding systematist who faces the problem.

As emphasized at the Vienna Congress, one of the fundamental points in nomenclature[6] "is to aim at fixity of names," and "in the absence of rule" (by which to bring this about) "established custom becomes law." However logical, therefore, Professor HITCHCOCK'S decision as to the type of *Danthonia* may be, it is essential for the sake of nomenclatural stability that the accepted custom of selecting the first species described (when the type is not indicated) be adhered to. Otherwise, as pointed out by Professor HITCHCOCK, some other botanist may say, "I favor selecting" *D. provincialis* DC. as the type.

Now *Festuca decumbens* is not only the first species described, but Professor HITCHCOCK fails to show that DE CANDOLLE did not consider it completely congeneric with his *D. provincialis*. It is provided with an awn, even though rudimentary, and, to quote, "it is evident that the author considered the awn to be one of the important distinguishing characters of his new genus." DE CANDOLLE'S suggestion that *Avena*

[4] The seeds of all the *Citrus* fruits examined showed an abundance of oxidases.

[5] The type species of *Danthonia*. BOT. GAZ. **57**:328. 1914.

[6] Vienna Rules, 35. 1905.

spicata should be included surely does not indicate that he regarded it as *more* representative of his *Danthonia* than *decumbens*, since his generic description provides for the latter ("awn sometimes long, sometimes rudimentary").

The adoption of *Avena spicata* L. as the type, therefore, is seen to be purely arbitrary, since such action is based on the present interpretation of the genus.—AVEN NELSON and J. FRANCIS MACBRIDE.

MATURATION IN VICIA

(PRELIMINARY NOTE)

The following preliminary note summarizes the results so far obtained in a study which has been temporarily interrupted. Although many details remain to be worked out, the following points seem clear.

In the somatic cells of *Vicia Faba* there are twelve chromosomes; two of them are about twice as long as the other ten. How this size difference arose is not known, but there is some reason to believe that each long chromosome may have been formed originally by the coherence of two ordinary ones.

In the early prophases of the heterotypic mitosis in the pollen mother cells, the chromosomes take the form of long slender threads (leptonema), which become paired side by side (zygonema). These double threads shorten and thicken (pachynema), the association of the two members of each pair becoming very intimate. The nature of this union has yet to be determined. Synizesis occurs during these prophase stages as a natural phenomenon.

At diakinesis there are six gemini; one of them is about twice as large as the other five, showing that the two large chromosomes seen in the somatic cells have paired with each other. At the first maturation division the members of each pair pass to opposite poles, bringing about the reduction. In the second, or homeotypic, mitosis all the chromosomes divide longitudinally, so that each microspore receives six chromosomes, five short and one long.

The megaspore mother cell has not been examined, but in the light of the above data on somatic and pollen cells it seems probable that similar phenomena occur in the maturation of the megaspore.

The results here recorded are of special interest in that they furnish further evidence in favor of the theory that the two chromosomes which pair and separate at the first maturation division come one from each parent, and are in some sense homologous.—LESTER W. SHARP, *University of Chicago*.

CURRENT LITERATURE

BOOK REVIEWS

Irritability of plants

The experimentation described in BOSE's new volume[1] is marked by excellence of methods and execution. The presentation is in direct, clear, and in many places elegant English. The findings are rendered quickly available by comprehensive but concise summaries at the end of each chapter and at the end of the volume. One regrets that this excellent work is marred here and there by oldness of viewpoint and lack of knowledge of physiological literature.

In his older researches BOSE used the optical lever with the photographic method of recording. This made the taking of automatic records tedious, with the possibility of relatively few records during the active season of the plant. It also forced experimentation in an abnormal condition for the plant, namely, continual darkness. The resistance offered by the recording apparatus, both because of weight of the arm and because of the friction of the writing point on the black surface, was another difficulty in the taking of automatic records. The author has overcome all of these difficulties by his new and ingenious resonant and oscillating recorders. He has designed arms that are about 0.01 as heavy as those used with the muscle nerve, and the oscillating or resonant recorder gives a series of dots on the black surface rather than a continuous line. This reduces the friction to a very small fraction of that of the continuous record. In the *Desmodium* leaflet the author has shown that a continuous line gives a great reduction in the magnitude of the movement, while none occurs with the new recorder. His new apparatus also makes possible the recording of periods as short as 0.001 of a second. One is convinced that BOSE is reaching the optimum of accuracy and delicacy in the matter of automatic records of plant response, and this is much needed, for many fewer such records exist for plants than for animals.

This excellent experimentation results in a number of things of interest to plant physiologists; in some cases confirming views already held; in others showing prevailing views at error; and in still others giving exact determinations of physiological critical time periods. BOSE shows that the sum of stimulus law holds for a number of stimuli, as has been shown to be the case for geotropism, heliotropism, etc. In subtonic condition of an organ the stimulus itself renders the organ more excitable to the same stimulus. He has apparently established beyond doubt that conduction of a stimulus in the

[1] BOSE, J. C., Researches on irritability of plants. xxxiv+376. *figs. 190.* London: Longmans, Green & Co. 1913.

petiole of *Mimosa* cannot occur over a zone that is dead or in a state of rigor, contrary to the claims of HABERLANDT and PFEFFER. As BOSE puts it, conduction of stimuli in this organ is a true "excitatory" or "physiological" process. His statement that plant physiologists universally hold that conduction of stimuli in plants is "hydromechanical" shows that he has entirely overlooked the generally accepted and much quoted classic of FITTING (1907) on the conduction of the light stimulus in the coleoptile of *Avena*. The author's findings in *Mimosa* do not, contrary to his statement, disprove a universally held view in plant physiology, but they merely show that the conduction of stimuli in this form is consistent with that in *Avena*, which probably represents the general situation for plants. He has shown that PFLUGER's laws of polar excitation in animal tissues hold for plant tissues, and supplements these by two more laws that hold for strong currents. This is only one of many illustrations that BOSE brings forward of fundamental likenesses in stimulus effects and response in plants and animals. Records are given of very accurate determinations of latent periods (the time elapsing between application of the stimulus to the pulvinus and the beginning of movement) in the leaves of *Mimosa* and other motile leaves, as well as exact measurements of the rate of conductivity of stimuli. In the primary pulvinus of *Mimosa* the shortest latent period found was 0.06 second, and the average 0.1 second. In subtonic but not in optimum condition of the organ, increased strength of the stimulus shortens, while in general fatigue prolongs, and a rise in temperature shortens this period. The maximum rate of conduction was that found in the petiole of *Mimosa;* it was 30 mm. per second. Conditions that shorten the latent period also increase the rate of conduction. In subtonic but not in optimum condition of the organ, previous application of the stimulus hastens the rate of conduction. Conduction takes place in both directions, but not always at the same rate. In *Biophytum*, for instance, the velocity is greater in the centrifugal than in the centripetal direction.

BOSE emphasizes that conduction of the stimulus proper is accompanied by a wave of contraction and of galvanometric negativity. This is contrasted with the wave of galvanometric positivity which passes over a stimulated non-living object or as a secondary effect of stimulation in the living organ, and which travels much faster than the primary stimulus effect. The author emphasizes this distinction between true "excitatory" or "physiological" conduction, accompanied by contraction and galvanometric negativity, and the "physical" conduction, marked by galvanometric positivity. This is good, but physiology is beyond the stage of stopping with such distinctions. It now recognizes that the effect and conduction of stimuli proper in the organism are physical also, undoubtedly more complex than the behavior in the non-living, but physical nevertheless. Two groups of facts, each in part and both together, probably fully explain the physics of the contractile wave with its galvanometric negativity. One group of workers[2] has produced much

[2] Science N.S. 37:959–974. 1913.

evidence that the living cells of the organism are surrounded by semipermeable membranes with a difference in electrical potential outside and inside the membrane, and that any stimulus causes an increase of permeability with its necessary contractile and electrical effects. Again, the behavior of colloids in the form of gels leads one to believe that hydration-changes accompany response to stimuli in the protoplasm, which always involve contractile and electrical effects. These are probably the physical factors entering into BOSE'S "physiological" response and conduction. With death (coagulation of the colloids) both the membranes and the colloids disappear, and with them the "physiological" response.

Many statements in the book show a lack of familiarity with the anatomy and physiology of plants. The author speaks repeatedly of depleted energy in the pulvinus of *Desmodium*, or of its receiving energy from without. This is rather vague in the light of what we know of the energy relations of plants. He applies systole and diastole to the pulvinus as he does to the heart, and compares the petiole of *Mimosa* with the nerve and the pulvinus with the muscle of a nerve muscle preparation. Both of these statements are made in spite of the fundamental differences in structure and function. The death point for plant cells is fixed, as if mysteriously, at 60° C., and death from a lower temperature is spoken of as being very different in nature. BOSE fails to recognize that death at supramaximal temperatures is a matter of coagulation of certain proteins, and that at any supramaximal temperature the time of its application is a function in the process. At temperatures lower than 60° C. the time must be longer. The reason that 60° C., rather than a lower or higher temperature, is found to be the death temperature in a given species, is because of his method of heating, namely, 1° C. rise in temperature per minute. A slower heating would give a lower death point, and a faster one a higher one. The reason for the constancy of this temperature for the different species studied is not so clear. BOSE should have been acquainted with LEPESCHKIN'S[3] work on this subject. It is of some interest that the death temperature is lowered by fatigue to 37° C., and by copper sulphate to 42° C. This emphasizes again the marked lability of certain cell proteins, and reminds one of LEPESCHKIN'S results showing that they are coagulable even by pressure.

BOSE is adding much to the subject of plant response, but his excellent methods could add much more if they were applied in the light of our modern knowledge of the physics and chemistry of living cells and of plant response in general.—WILLIAM CROCKER.

Biology and capillary analysis of enzymes

GRÜSS[4] has brought together in book form the results of his work on enzymes, which has extended over a period of several years. Some of his con-

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[3] Ber. Deutsch. Bot. Gesells. **30**:703–714. 1913.

[4] GRÜSS, J., Biologie und Kapillaranalyse der Enzyme. 8vo. pp. vi+227. *col. pls. 2. figs. 58.* Berlin: Gebrüder Borntraeger. 1912. *M.* 16.

clusions are a decided departure from the usual conceptions regarding enzyme action. Probably the most striking departure is the assumption that different enzyme actions may be attributed to different atomic groups of the same enzyme; for example, malt diastase is also capable of cytase and peroxidase action. Many of the data upon which his conclusions are based are drawn from capillary analyses. This method for the separation of soluble substances the author has very ingeniously modified and elaborated for his particular purposes. With modifications excluded in special instances and stripped of details, the general method may be described as follows: A few drops of the enzyme mixture to be analyzed are placed upon filter paper. By capillary attraction a circular field is formed in which the dissolved substances arrange themselves in more or less distinct zones. In the case of colloidal substances, a greater spreading and separation is effected if a water ring is first placed upon the filter paper. Diffusion forces then come into play as well as capillary attraction. Diffusion is rendered more active if the solution to be analyzed contains a little glycerine. After spreading ceases, the capillary field is cut into sectors and each treated with a different reagent which gives a color reaction for a specific enzyme. The diastase test, for example, is carried out by placing firmly a sector of the capillary field upon a similar piece of filter paper moistened with a solution of soluble starch. After the diastase present in the sector of the capillary field has had sufficient time to hydrolyze the starch, the starch paper is treated with iodine solution. The presence and extent of the diastase in the field are then shown by a white area bordered by the blue-violet color of the starch iodine reaction. For peroxidase a sector is treated with guajak+H_2O_2; for oxydase, violamin is employed, etc. The treated sectors are all brought together to form the "chromogram," which shows the distribution or relative spreading of the different enzymes in the capillary field. If the same area is capable of more than one enzyme action, the author believes that it is due to a complex enzyme and not to the coexistence of different independent enzymes. He also believes that he has further confirmed his theory by showing that certain complex enzyme actions of capillary analysis appear also simultaneously in germination and are simultaneously destroyed by a given treatment of the enzyme preparation.

The enzymes of various embryos and endosperms, potato tubers, fungi, yeast, and latex were extensively studied with the aid of the capillary method, together with microchemical tests. The following are a few of the author's most important biological inductions: The enzymes and their anti-enzymes form systems in which the members oscillate around an equilibrium position. Upon these systems, which deserve the designation *Träger des Lebens*, rests the synthesis and disintegration of the cell substances. The most important system is the group of oxidizing enzymes: oxidase+peroxidase+antioxidase (hydrogenase). Antioxidase is simply a general term indicating any inhibition of an oxidase action. The antioxidase function is probably exercised by many different kinds of substances which are not all of an enzyme nature, since

in many cases they are changed by their own activity and are not destroyed by hot alcohol. The enzyme nature of hydrogenase, however, is not to be doubted. It performs its function by liberating nascent hydrogen which unites with the oxygen carried by the oxidase to form water or hydroxyl groups; the latter can be employed for the formation of metabolic products. By these formations the oxidation of other substances is prevented. "Reductase" is a *contradictio in adjecto*. "Catalase" is merely a term to designate the decomposition of H_2O_2, and its place in the category of enzymes is extremely hypothetical. Amylocoagulase is the anti-enzyme of diastase in the sense that its action is just the opposite of that of diastase. The coagulase may be entirely obscured by the action of diastase, but it cannot be reversed. Cytocoagulase is another synthetic enzyme which acts in opposition to cytase.

The chief value of the book lies in the mass of experimental details and the ingenious methods devised for the study of enzymes. It will find its greatest usefulness among those especially interested in enzymes, as the treatment is rather technical.—CHAS. O. APPLEMAN.

Field manual of trees

An addition to the already numerous tree manuals has come[5] in the form of a thin pocket volume, 4.5 by 7 inches, bound in flexible leather, making it particularly convenient for field use. It is designed to include all the native and many of the introduced species north of Virginia and Kentucky and east of the prairie region. The features which recommend this manual are the numerous keys, all of which appear to be accurate and rather simple. They include separate ones for the genera based on (1) summer condition, (2) winter condition, (3) flowers, and (4) fruit, as well as keys for species under each genus and a general classification of the wood of the trees. The descriptions of the species seem to be good, but it is certainly unfortunate that such well known trees as the black and choke cherry should appear under such scientific names as *Prunus virginiana* and *P. nana* respectively, and that the flowering dogwood should be removed from the genus *Cornus*. Whatever may be the arguments of the systematist for such a course, it is certainly likely to lead to confusion, especially in a manual where no synonyms are given. One also misses the illustrations which have been a prominent feature of many recent volumes on trees.—GEO. D. FULLER.

MINOR NOTICES

Botanical researches of the Carnegie Institution.—The annual report[6] for the past year gives an idea of the various lines of research completed and

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[5] SCHAFFNER, JOHN H., Field manual of trees, including southern Canada and the northern United States to the southern boundary of Virginia, Kentucky, and Missouri, westward to the limits of the prairie. Columbus (Ohio): R. G. Adams & Co. 1914. 16mo. pp. 154. Cloth $1.25. Limp leather $1.75.

[6] MACDOUGAL, D. T., Annual report of the director of the department of botanical research. Carnegie Institution of Washington, Year Book No. 12 for 1913:57–87. 1914.

in progress at the Desert Laboratory of the Carnegie Institution and of the reports that are soon to appear. The activities are so numerous and the scientific staff so large that all cannot be mentioned in a brief notice, but some are especially worthy of note.

Studies upon the Salton Sea by MACDOUGAL and several of his associates since 1907 have been very extensive, and include investigations of the soil and rock deposits, yearly analyses of the water as the sea continued to dry up, studies of the action of this water upon vegetable tissues, of the destruction of the flora upon the submerged areas, and of the revegetation of the sterilized islands and beaches. These investigations have given remarkable results from the unique phenomena of this semi-arid region. A few of the reports have appeared and more are to be published very soon. The modification of plants under the specialized conditions of the Salton area are being studied, as well as the effects of various climatic complexes. W. A. CANNON is continuing his investigations of the root characters of desert plants and has also extended his activities to include the roots of trees grown in the coastal climate of California. FORREST SHREVE and his associates have made critical studies of transpirational behavior in many very diverse plants, and also of growth rate and winter temperature in the Santa Catalina Mountains. H. A. SPOEHR has been investigating the photolytic effect of the blue-violet rays and their variations in polar radiation; while B. E. LIVINGSTON, with several assistants, has been making exact studies of the water relations of plants, including both the aerial and the soil conditions. The fruit development of the Cactaceae has been investigated by D. S. JOHNSON and the relationships and distribution of the same family by N. L. BRITTON and J. N. ROSE.—GEO. D. FULLER.

Annals of the Bolus Herbarium.—The first South African journal of botany has just made its appearance under the above title. The editor is Professor PEARSON of the South African College. The first number of the journal contains 40 pages, and it is announced that two parts will probably appear each year, and that four parts will constitute a volume. The reasons for a South African journal of botany are given, and seem to be well taken. The field for botanical investigation in South Africa is very large and but sparsely occupied, and the number of those directly or indirectly interested in botanical work is increasing. The new journal, as its name implies, will be mainly concerned with the botanical work inspired by Dr. BOLUS and with investigations conducted in connection with the Bolus Herbarium. This means that its field will be chiefly the taxonomy, ecology, plant geography, and economic botany of South Africa. There will also be included articles that may prove of assistance to those engaged in teaching botany. In a certain sense the journal will also be an organ of the newly established National Botanic Garden.

The first number contains the following papers: "On the flora of the Great Karasberg," with an introduction by PEARSON giving an account of the region, and a list of plants by F. and L. BOLUS and R. GLOVER; "Novitates

Africanae," in which a new genus (*Pillansia*) of Iridaceae is described by L. BOLUS; and "Key to the flora of the Cape Peninsula," by F. and L. BOLUS. —J. M. C.

Annals of the Missouri Botanical Garden.—The present year has been prolific in the appearance of new botanical journals. To the *American Journal of Botany* and the *Annals of the Bolus Herbarium* is now added the *Annals of the Missouri Botanical Garden.* The new journal is a quarterly, the first number being dated March 1914. The journal will provide for the printing of scientific papers which formerly constituted a large part of the annual report of the Missouri Botanical Garden. It will contain only scientific contributions from members of the staff of the Garden, from the faculty and graduate students of the Washington University, and from visiting botanists doing all or part of their work at the Garden. The first number contains the following papers: "The effect of surface films and dusts on the rate of transpiration," by B. M. DUGGAR and J. S. COOLEY; "Some pure culture methods in the algae," by JACOB R. SCHRAMM; "The identification of the most characteristic salivary organism and its relation to the pollution of air," by AUGUST C. NOLTE; "The *Polyporaceae* of Ohio," by L. O. OVERHOLTS.—J. M. C.

The fresh-water flora of Germany, Austria, and Switzerland.—Part 1 of this series of brochures has appeared.[7] The five previous parts have been noticed in this journal.[8] The present part completes the flagellates, the other groups of which were presented by PASCHER and LEMMERMANN in part 2. The compact size, excellent illustrations, and well considered analytic keys continue to be features of this excellent work.—J. M. C.

NOTES FOR STUDENTS

Color inheritance.—Continuing his excellent studies on *Melandrium* (*Lychnis*), SHULL[9] has made a great advance in our knowledge of the inheritance of leaf pigments of the chlorophyll and the carotin-xanthophyll groups. With his characteristic care, the author came to his conclusions only after a very large number of hybrids properly synthesized for the tests desired had been made. The color wheel was used as an aid to the classification of the individuals wherever it was deemed necessary. BAUR's discovery of a general factor for chlorophyll formation (*Z*), without which plants are free from chlorophyll, is confirmed; but his idea that the gene *Z* produces yellow pigment is not supported. Assuming the presence of unanalyzed genes *XX*, then typical

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[7] PASCHER, A., Die Süsswasser-Flora, Deutschlands, Österreichs, und der Schweiz. Part 1. Flagellatae, by A. PASCHER and E. LEMMERMANN. pp. 138. *figs. 252.* Jena: Gustav Fischer. 1914.

[8] BOT. GAZ. **56**:233. 1913; **57**:335. 1914.

[9] SHULL, G. H., Über die Vererbung der Blattfarbe bei *Melandrium.* Ber. Deutsch. Bot. Gesells. **31**:40–80. 1914.

dark green plants of *Melandrium* are *XXZZYYNN*. This was shown by crosses between two light green types, *chlorina* and *pallida*. The F_1 generation was the typical dark green, while the F_2 generation consisted of dark green and light green in the ratio 9:7. Among the light green individuals both *chlorina* and *pallida* plants could be recognized, but just what characters the plants with the formula *XXZZyynn* possessed was not determined.

Three cases of non-mendelizing leaf variegation are also described:

1. This case of variegation was a chimera made up of typical dark green and of pure white areas. Seed from green branches or from white branches produced progeny exactly like the mother, no matter what characters were possessed by the male parent.

2. These plants are called *chlorinomaculata*, because they are dark green spotted with the "chlorina" type of green. The transmission of their characters is not yet entirely clear. The progeny of a female plant crossed with pollen from flowers of different colored branches gave the following results: from variegated branches came green, variegated, and chlorophyll-free plants; from green branches came only green plants; and from *chlorina* branches came only chlorophyll-free plants.

3. These plants were of the yellowish *aurea* type. They were crossed with many other forms, but the results are somewhat complex, and the author does not commit himself definitely on their analysis. He thinks that possibly this may be a case of infectious chlorosis. He says: "While chlorosis of *Abutilon* and other Malvaceae is transmitted neither through the male nor the female gametes, this *aurea* character is carried by a part of the gametes of both kinds." It seems to the reviewer that if this phenomenon is indeed one of infectious chlorosis, the small number of *aurea* plants of the filial generations might easily be due to reinfection.—E. M. East.

Statistical methods in phytogeography.—In his attempts to obtain more exact data regarding the distribution of the various elements of alpine flora, Jaccard[10] has developed certain statistical methods that have not only revealed several interesting facts regarding the vegetation of the Alps, but promise to be equally serviceable in the investigation of other areas. Having made a census of the areas to be compared, in this instance similarly situated localities of approximately the same area in various parts of the Alps, he applies for the analysis of his results his *coefficient of community* (C.c.), that is,

$$\frac{\text{No. of species common to two districts} \times 100}{\text{Total no. of species in the two districts}} = \text{C.c.}$$

For alpine meadows at an altitude of 1900 m., several interesting results were manifest, such as: (1) the fact that the value of C.c. does not depend upon floral richness, but upon the ecological characters of the areas studied; (2) the

[10] Jaccard, Paul, The distribution of the flora in the alpine zone. New Phytol. 11:37–50. 1912.

alpine flora is extremely diverse in floristic composition; (3) the rare species are most numerous and the common species least numerous (this does not apply to number of individuals); and (4) the C.c. is generally higher for contiguous than for distant areas. This last result is strikingly demonstrated in the study of the vegetation upon some alpine gravel areas[11] of the Alps. In this latest report JACCARD also makes extensive use of his generic coefficient (*coefficient générique;* C.g.), that is,

$$\frac{\text{No. of genera} \times 100}{\text{No. of species}} = \text{C.g.},$$

with instructive results. This coefficient is shown by studies of both alpine and dune floras to vary inversely with the variety of ecological conditions in the areas compared, and hence in alpine areas the value of C.g. increases with altitude, while in some dune areas of Belgium, the C.g. is greatest (100) under the excessive and narrow ecological limits of the moving dunes, and least (73) under the more varied ecological conditions of the pannes.

These analyses lead the author to the following among other conclusions: (1) The distribution of plants (at least in the alpine zone) is a resultant of the combined action of three orders of factors; (*a*) ecological, (*b*) biological, or degree of adaptation, and (*c*) sociological, or competition between species; and (2) the action of these factors has resulted in (*a*) an eliminative selection of species, and (*b*) a distributive selection determining the number of individuals and the nature of associated species.—GEO. D. FULLER.

Tetraspore formation in Nitophyllum.—Since it has been shown that in *Polysiphonia* and *Dictyota* the reduction of chromosomes occurs during the formation of tetraspores from the tetraspore mother cell, it is natural to inquire what cytological conditions obtain during tetraspore formation in those red algae which have multinucleate cells. One might guess that the tetraspore mother cell is uninucleate, or that it is multinucleate and all the nuclei except one disorganize. Soon after YAMANOUCHI'S paper on *Polysiphonia* appeared, SVEDELIUS examined *Martensia*, one of the Delesseriaceae. The tetraspore mother cell is multinucleate and, as the cell enlarges, the nuclei multiply until there are about fifty. Then all but one disorganize, and four tetraspores are formed. The fixing did not allow a detailed cytological study. Recently, however, SVEDELIUS[12] secured well fixed material of *Nitophyllum punctatum*, a member of the same family, and succeeded in working out the cytological situation. The thallus is prevailingly one cell thick, but when tetraspores are so formed, it becomes three or four cells thick. The tetraspore mother

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[11] JACCARD, P., Étude comparative de la distribution florale dans quelques formations terrestres et aquatiques. Rev. Gén. Botanique **26**:5–21, 49–78. 1914.

[12] SVEDELIUS, N., Über die Tetradenteilung in den vielkernigen Tetrasporangiumanlagen bei *Nitophyllum punctatum*. Ber. Deutsch. Bot. Gesells. **32**:48–57. *pl. 1.* 1914.

cell has several nuclei, and nuclear division continues after the mother cell has become recognizable, but the number of nuclei seldom exceeds a dozen. In the vegetative mitoses the chromosomes are organized directly from the reticulum, but in the tetraspore mother cell the formation of chromosomes is preceded by a typical spirem stage. After the number of nuclei reaches about a dozen, some begin to disorganize, but several may develop spirems and continue up to a typical metaphase of the heterotypic mitosis; then one nucleus continues and all the rest disorganize. The details of the division of the successful nucleus and the organization of tetraspores agrees fully with the account given by YAMANOUCHI for *Polysiphonia*.—CHARLES J. CHAMBERLAIN.

Distribution and development of an Ohio flora.—A region in southern Ohio designated as "Sugar Grove" and situated at the end of a long lobe of Merriam's Alleghenian floral area, is regarded by GRIGGS[13] as remarkable on account of its being the meeting place of many very diverse floras. He has mapped six such groups, consisting of (1) Alleghenian plants, 39 species; (2) Appalachian plants (northern), 14 species; (3) Appalachian plants (southern), 12 species; (4) Carolinian plants, 12 species; (5) Mississippian plants, 15 species, and (6) northern plants, 9 species, and finds Sugar Grove upon extreme limits of each group. The data for establishing the range of these 121 plants are scanty, as the author admits, but that many of these species are near the limit of their distribution seems quite evident. This has led to an investigation of the behavior of many species at the edges of their range,[14] and almost every possible phase of behavior is found to be exhibited. So varied appear the responses that it would seem difficult to draw any general conclusions, although the author decides that the limits of species reaching the edges of their ranges near Sugar Grove are not fixed, but are changing, and that plants of boreal affinity are apparently being displaced by others from the west and south, a continuation of the floristic movements following the glacial period. *Tsuga canadensis* is cited as an example of such movements, and GRIGGS asserts that it is now found in southern Ohio only because it has not been completely displaced by the post-glacial flora, and occupies its habitats simply because within them the invading hardwood forest has not had so good an opportunity to gain a foothold as elsewhere. This explanation seems plausible, but the reviewer cannot regard the case as proved.—GEO. D. FULLER.

Prothallium of Equisetum.—KASHYAP[15] has investigated the prothallium of *Equisetum debile* as it grows in abundance in the vicinity of Lahore, India.

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[13] GRIGGS, R. F., Observations on the geographical composition of the Sugar Grove flora. Bull. Torr. Bot. Club **40**:487–499. 1913.

[14] GRIGGS, R. F., Observations on the behavior of some species at the edges of their ranges. Bull. Torr. Bot. Club **41**:25–49. 1914.

[15] KASHYAP, SHIV R., The structure and development of the prothallium of *Equisetum debile* Roxb. Ann. Botany **28**:163–181. *figs. 45*. 1914.

This first investigated Asiatic species proves to be of great interest, as the following summary of results will show. The prothallium is exceedingly variable in its early stages, and of special interest is the occasional occurrence of a "primary tubercle" comparable to that of *Lycopodium cernuum*. The lobes of the prothallium are always erect and very close together, both in nature and in a darkened room, so that this upright position holds no relation to the amount of light. One of the striking features of the prothallium is its radial symmetry, which disposes of the claim that the fundamental difference between the prothallium of *Lycopodium* and of *Equisetum* is that the latter is not radial, but dorsiventral. The prothallium of this species also proves to be much larger than the largest that have yet been found in the genus. There are no male prothallia, but sometimes prothallia do not produce antheridia, and therefore are female. The antheridia resemble those of *Lycopodium* in position, general structure, and paraphyses. The archegonium has a single neck canal cell, which is also a feature of resemblance to *Lycopodium cernuum*. The author reaches the general conclusion that there is a clear affinity with the prothallium of *Lycopodium cernuum*, and that there is no more difference between the two prothallia than is already known to occur among the species of *Lycopodium*.—J. M. C.

The embryogeny of Balanophora.—The researches of TREUB and LOTSY on the embryogeny of the Balanophoraceae are well known. In *Balanophora* they found the four nuclei in the antipodal end of the sac, and also the synergids and egg degenerating as soon as the sac reached the fertilization stage; the remaining micropylar polar nucleus gave rise to a cellular endosperm, from one of whose cells the embryo developed.

ERNST'S studies on the embryogeny of saprophytic forms led him to suspect that there might be a simpler explanation of the origin of the embryo of *Balanophora*. A reinvestigation[16] confirmed the previous accounts of the origin and development of the embryo sac, the degeneration of the antipodals and the synergids, and the formation of a cellular endosperm from the micropylar antipodal; but it also showed that the embryo is developed from the egg. The development, however, begins late, after the egg is surrounded by cellular endosperm, and it was this behavior which misled both TREUB and LOTSY. There is no fertilization in either *Balanophora globosa* or *B. elongata*. Both are parthenogenetic. The development of the sac shows the diploid number of chromosomes.—CHARLES J. CHAMBERLAIN.

Experimentation in plant geography.—MASSART[17] has given emphasis to the fact that plant geography has hardly kept abreast of other branches of

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[16] ERNST, A., Embryobildung bei *Balanophora*. Flora **106**:129–158. *pls. 1, 2.* 1913.

[17] MASSART, J., Le role de l'éxperimentation en géographie botanique. Rec. Instit. Bot. Léo Errera **9**:68–90. 1913.

biological science on account of the lack of experimental methods of investigation, and he has consequently undertaken to indicate certain classes of problems which are demanding experimental solution. Certain species, for example, occupy habitats so diverse that there seems legitimate ground for doubting whether the different forms are specifically identical. Amphibious species of *Polygonum* furnish striking examples of form variation and specific relations only to be determined by extensive experimental cultures. Here he recommends that "accommodations" be limited to transformations of individuals in response to changed environment, and "adaptation" to those of species, the former not being hereditary, but the latter being transmitted to offspring. The author also urges the investigation by experimental methods of all examples of apparent multiplicity of origin of species, and of natural hybrids and mutations.—GEO. D. FULLER.

Vascular anatomy of the Cycadophytes.—Miss BANCROFT[18] has traversed the evidence of the relationship between the vascular anatomy of the Cycadofilicales and the Cycadales. The particular vascular type of Cycadofilicales from which the cycad line has developed is the problem to which at least three answers have been given. SCOTT favors origin from the *Lyginodendron* type; WORSDELL from the *Medullosa* type; and Miss DE FRAINE from a situation represented by *Sutcliffia*, which rather mediates between the two preceding views. Miss BANCROFT has done good service in bringing the whole evidence of this critical situation together, and has reached the conclusion that the *Lyginodendron* and *Medullosa* types have arisen from a common stock, and that from this stock the cycad line has arisen, touching very close to the simpler forms of the *Medullosa* group. When competent opinion differs as to the particular origin of a given group, it will generally be found that the dispute can be arbitrated by referring all of the groups to a common origin.—J. M. C.

Leaf and root.—Many find difficulty in identifying a stem in some vascular plants. CHAUVEAUD[19] diagrams the first leaf and root of *Ceratopteris thalictroides* and calls the combination a *phyllorhiza;* the second leaf with its root constitutes the second *phyllorhiza*, etc. The fused portions are commonly called the stem. *Cordyline anstralis* presents a similar condition. In dicotyls, the first two phyllorhizas are not separated in time or space; they have a fused portion, the stem, and a common root. Why it should make any difference as to whether the fusion exists from the start, or occurs a little later, is not entirely clear. However, it must be admitted that even in some plants with two cotyledons, like the cycads, the two categories, leaf and root, seem sufficient for the

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[18] BANCROFT, NELLIE, Pteridosperm anatomy and its relation to that of the cycads. New Phytologist **13**:1 and **2**:41–68. *figs. 20.* 1914.

[19] CHAUVEAUD, GUSTAVE, La constitution et l'évolution morphologique du corps chez les plantes vasculaires. Compt. Rend. **158**:343–346. *figs. 8.* 1914.

seedling, and that the stem category is rather artificial.—CHARLES J. CHAMBERLAIN.

An arctic-alpine plant association.—Upon the "snow-flush," a substratum deposited on gentle slopes or flats by streamlets of snow water and composed of fine snow-dust material, there develops a characteristic association or succession of associations, recently described by SMITH.[20] He indicates its occurrence on Ben Lawers and cites the work of others, notably that of SCHRÖTER, RÜBEL, and BROCKMANN-JEROSCH, concerning its development upon the Alps. Pioneer algae are succeeded by a thick mat of the liverwort *Anthelia juratzkana*, which gives character and name to the association. *Polytrichum* sp. follows and is succeeded by *Salix herbacea*, *Alchemilla*, *Gnaphalium*, and other alpine plants, the floristic composition of the later stages varying in different localities.—GEO. D. FULLER.

Plant geography of the heights of Hautie.—ALLORGE[21] has made a floristic study of a plateau 15 by 10 kilometers in area, situated northwest of Paris at the confluence of the rivers Seine and Oise. The elevation of the plateau is about 190 meters, and it exhibits a considerable diversity of soil, with comparatively natural vegetation. The associations have been segregated according to the chemical nature of the soil, and that of the calcifuges is found to be most conspicuous and to cover almost the entire top of the plateau. The regional affinities of the flora are examined and shown to be chiefly western, although the area also seems to be a rather notable meeting ground of certain northern and southern forms.—GEO. D. FULLER.

Nitrite assimilation.—KOSSOWICZ[22] has found that molds (*Aspergillus niger*, *Penicillium glaucum*, *Mucor Bodin*, and others) can readily assimilate nitrite when it is the only source of nitrogen. It is important that by the most delicate test (NESSLER'S method), HN_3 could not be detected in the cultures except in two instances, and in these only after long cultural periods (26 days). The nitrite-ion can evidently then be directly assimilated without the intermediate production of NH_3.—E. M. HARVEY.

A cytological life cycle.—In a series of diagrams based upon the life history of the fern, GRIGGS[23] presents current notions as to the behavior of chromosomes in the sporophyte and gametophyte, and also during fertilization and reduction. While the illustration should not be pressed too far, the diagram will be useful for didactic purposes.—CHARLES J. CHAMBERLAIN.

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[20] SMITH, W. G., Anthelia: an arctic-alpine plant association. Scot. Bot. Rev. **1**:81–89. 1912.

[21] ALLORGE, A.-PIERRE, Essai de géographie botanique des hauteurs de l'Hautie et de leurs dépendence. Rev. Gén. Botanique **25**:417–431, 472–493. 1913.

[22] KOSSOWICZ, ALEX., Nitritassimilation durch Schimmelpilze. Zeitschr. Gärungsphysiol. **3**:321–326. 1914.

[23] GRIGGS, R. F., A cytological life cycle. Ohio Naturalist **13**:142–145. *pl. 6.* 1913.

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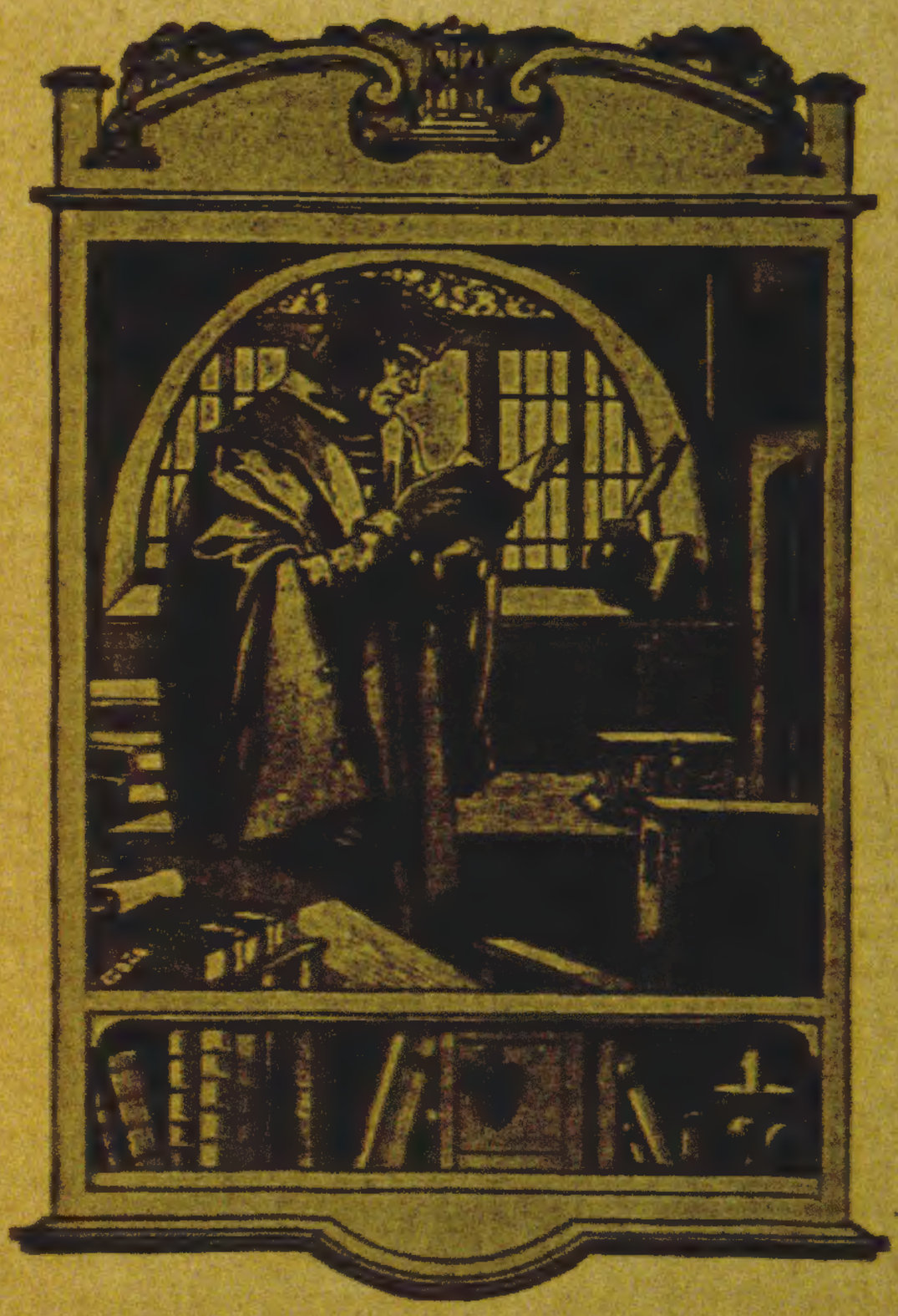

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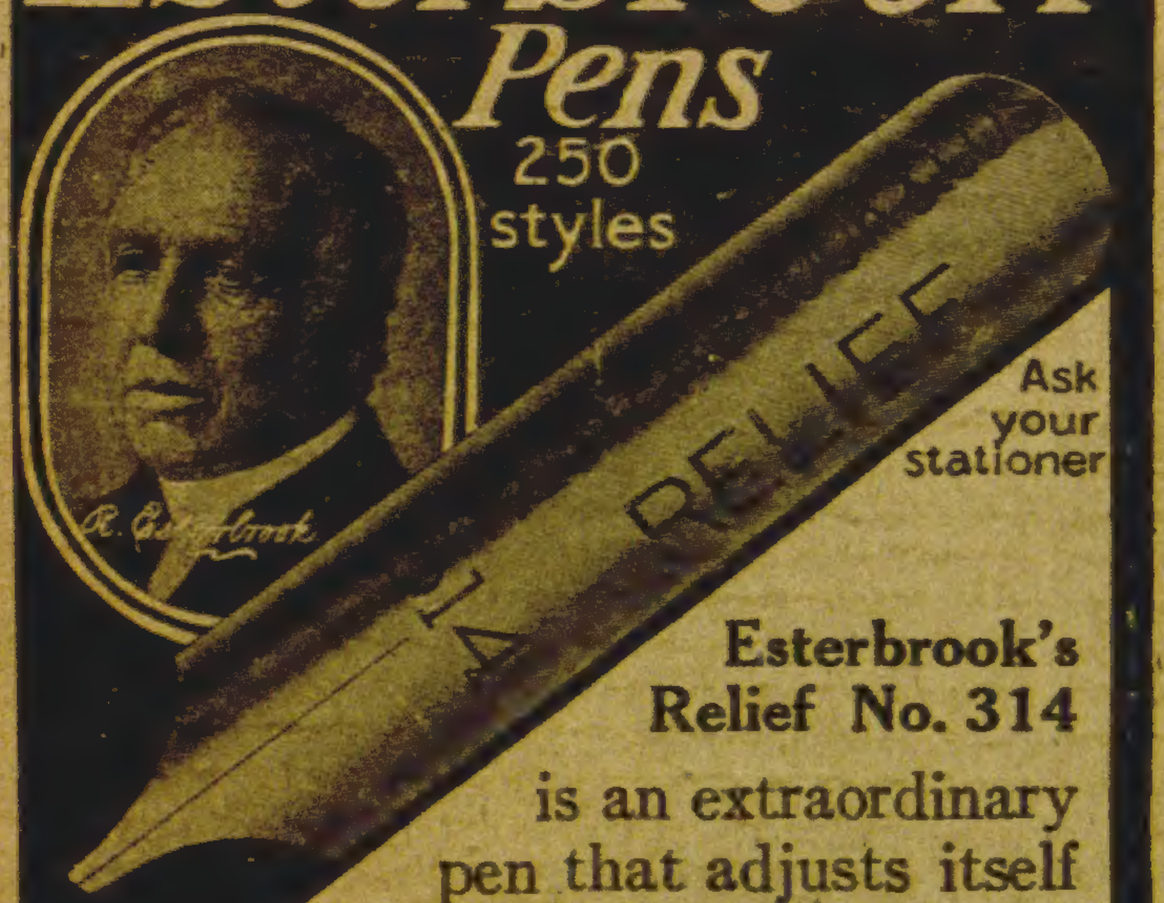

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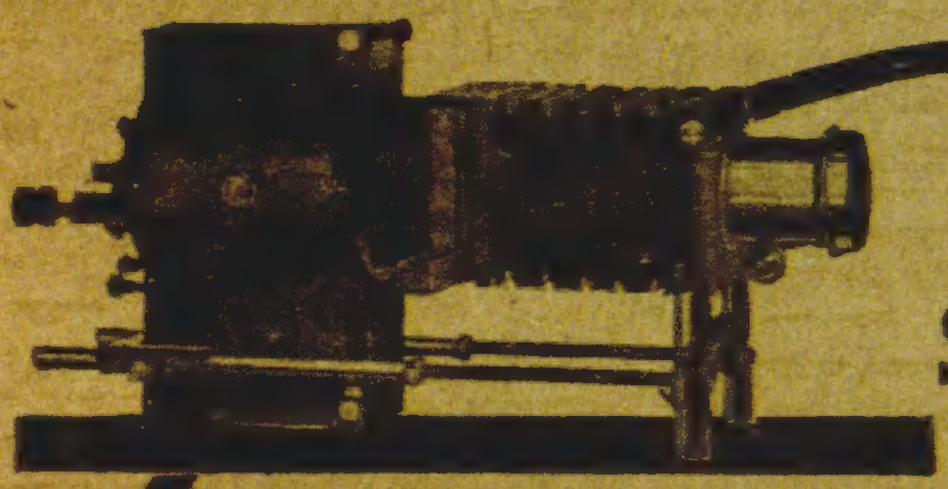

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